Drug effects on melanoma

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DRUG EFFECTS ON MELANOMA

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The essence of life is statistical improbability on a colossal scale.

– Richard Dawkins

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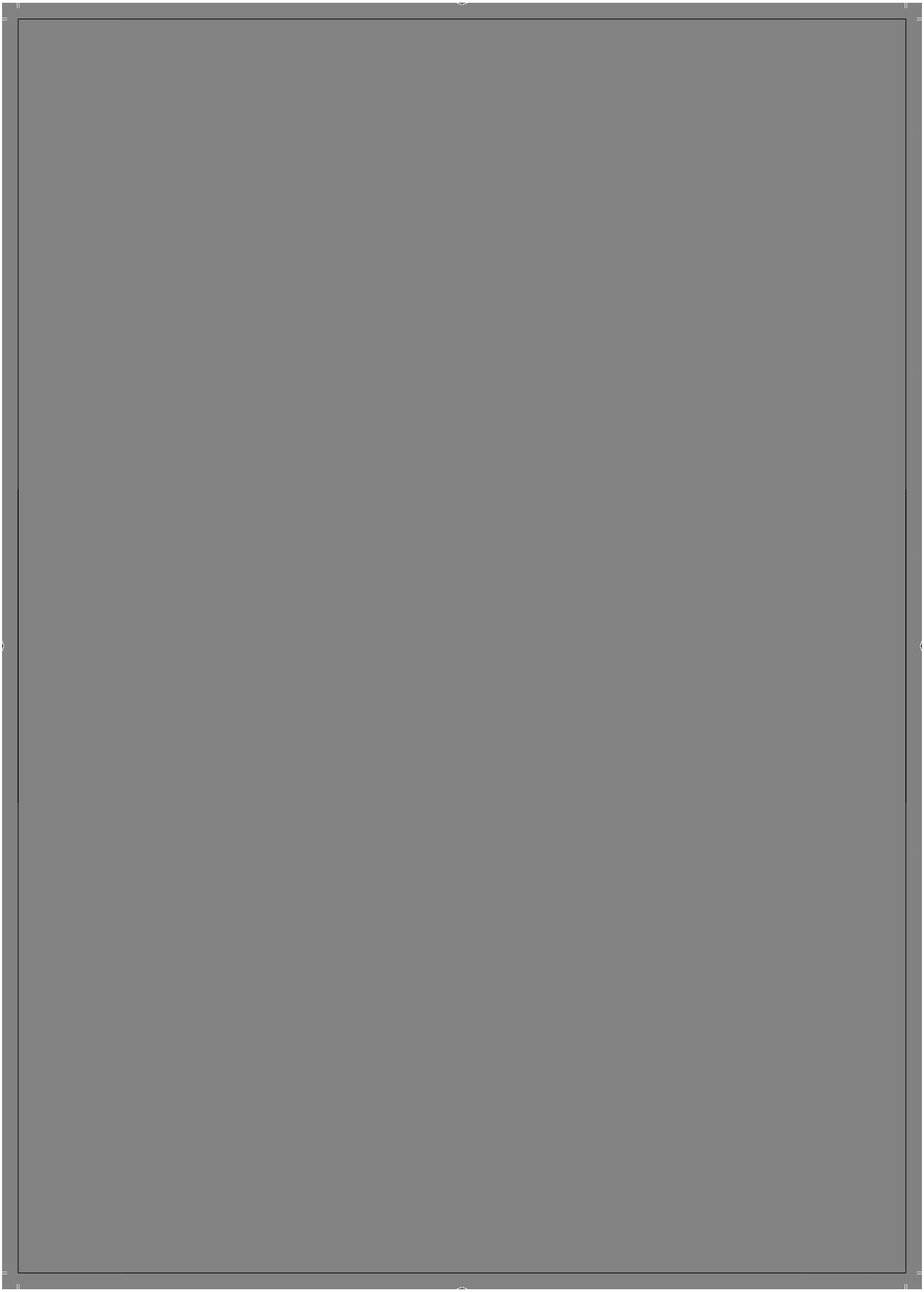

Chapter 1

Introduction and scope of the thesis

Introduction and scope of the thesis

Melanoma is the most aggressive form of skin cancer and is responsible for over 70 percent of skin cancer deaths. [1] Melanomas develop from malignant melanocytes. The gross majority of melanomas occur in the skin, the so-called cutaneous melanomas (CM). Melanoma incidence is among the top ten of leading cancer sites in the United States (US) with a fifth place for men and a sixth place for women. [1] Moreover, based on the years lost to cancer, melanoma would merit a higher ranking because relatively young people are affected by this malignancy. [2-4] Among Caucasian populations in Northern and Western Europe, melanoma incidence rates are increasing steadily by at least three percent each year. [5]

Melanoma prognosis depends on the stage at diagnosis. Melanoma staging is performed according the validated and internationally standarized Melanoma Staging System of the American Joint Committee on Cancer (AJCC). [6] This AJCC melanoma staging system is based on the TNM criteria; that is, thickness of the tumor (T), extent to which it has spread to the lymph nodes (N), and extent to which it has metastasized to other parts of the body (M). Tumor thickness, also referred to as Breslow's thickness is one of the strongest prognostic factors [6] and is measured from the skin surface until the deepest point of invasion as described by Alexander Breslow in 1970 [7]. Other factors that predict poor prognosis include advanced age at diagnosis, male gender, ulceration, race, anatomic site (trunk, head-neck region, extremities), and certain histogenetic subtypes, such as acral melanoma. The histopathological subtypes are classified according to the World Health Organization Classification of Tumours. [8]

Often CM are diagnosed at an early stage while the disease is still confined to the local site. For these patients, prognosis is favorable with 5-year relative survival proportions of 98.7 percent in the United States. In contrast, if the disease has spread regionally or in case of distant metastasis, 5-year relative survival proportions drop to 65.2 and 15.3 percent, respectively. [1] For these advanced stages of melanoma, effective treatment options are lacking [9], except may be surgical excision for localized metastasis. In spite of this lack of effective treatment options for advanced melanoma, melanoma mortality rates seem to be stabilizing or (slightly) decreasing. [10] In summary, overall melanoma incidence rates are increasing while mortality rates are stable or decreasing.

In rare cases, melanomas can also arise at noncutaneous sites such as primary melanomas of mucous membranes, the uvea or choroid of the eye, the meninges, or in organ tissue. [11] Due to their rarity, reliable estimates of the incidence and survival rates of such extracutaneous melanomas (ECM), e.g., from population-based registries, are sparse. Establishing the incidence rate of ECM, possible time trends in this incidence and the relative survival of ECM patients in The Netherlands, is a *first objective* of this thesis.
In **chapter 2** we will determine (trends in) the incidence rates of ECM in the Netherlands Cancer Registry. Additionally, we will present 5-year relative survival proportions among ECM patients in this chapter.

As mentioned earlier, melanoma mortality rates are stable or decreasing, while melanoma incidence rates are increasing. Since, additionally, melanoma is usually diagnosed in patients of a relatively young age [2-4], overall, the total number of patients suffering from melanoma is accumulating. Consequently, the total burden of melanoma is assumed to be increasing among Caucasian populations. Indeed, evidence from the US and Belgium has also suggested an increase in the burden of cutaneous melanoma. [3,12] Recent European data estimating (trends in) the different measures of the burden of CM, such as incidence rates, mortality rates, the prevalence, the number of years lost due to disability (YLD), and the number of years of life lost due to premature mortality (YLL), are sparse. The *second objective* of this thesis is to estimate of the burden of melanoma for the Dutch population. In **chapter 3** we will present estimates of the burden of melanoma in The Netherlands.

As the overall burden of melanoma is increasing; prognosis strongly depends on the stage at diagnosis; and, most importantly, effective treatments for advanced stages are lacking, there is a high potential benefit for the prevention of melanoma. However, most of the established risk factors for melanoma, such as fair skin type, freckles, light eye color, older age, history of sun burns, clinical atypical nevi, prior melanoma, and family history of melanoma, are not amenable to intervention. Only sun burns and sun exposure are, at least in theory, amenable. Indeed, sun protection measures are part of melanoma prevention programs. In some high risk countries, such as Australia, comprehensive sun protection programs have been implemented over a decade ago and sun screen use is widely promoted to the general public. These public health campaigns have increased awareness on skin cancer and the adverse events of excessive sun exposure, but failed to change the sun exposure behaviour in the general population which is referred to as the so-called 'knowledge-behaviour gap'.

Lack of behavioral changes and possibly also the increased awareness explain why the incidence of melanoma in Australia is still increasing. [13] Therefore, alternative approaches in melanoma prevention, such as chemoprevention, should be considered for high risk populations. Chemoprevention, as defined by Sporn and colleagues, is the use of natural or synthetic drugs to reverse, suppress, or prevent premalignant molecular or histological lesions from progressing to invasive cancer. [14]

Ideal candidate drugs for chemoprevention should have additional major health benefits, few (long-term) adverse events and would be inexpensive. Several drug classes, such as statins, aspirin, non-steroidal anti-inflammatory drugs (NSAIDs) and angiotensin-converting enzyme inhibitors (ACE inhibitors), have been suggested to be of interest in melanoma chemoprevention. [15-17] However, it is unclear which of these and other candidate drugs for melanoma chemoprevention have the potential to be useful and safe. Therefore, the *third and main objective* of this thesis is to explore which candidate chemopreventive drugs could be beneficial in melanoma and which drugs may be unfavourably associated with the incidence or progression of melanoma.
In **chapter 4** we will perform a qualitative review on a subset of the literature available on melanoma chemoprevention on these potential chemopreventive drugs. We will define this subset of the scientific literature with a systematic literature search in Medline, Embase, Web of Science, and The Cochrane Library.

To further explore if drugs have a chemopreventive effect on melanoma in humans, one could use several research designs, such as a prospective randomized controlled trial (RCT), prospective cohort study, retrospective cohort study or a case control study for instance by means of telephone surveys or by the use of pharmacy databases. However, in research practice, the choice of the study design is limited because one needs sufficiently long follow up and large numbers of participants to show chemopreventive effects on melanoma, a relatively rare malignancy that develops over long time periods. In addition, research funds are limited, and retrospective collection of drug exposure by telephone survey is time-consuming and may even be unreliable. For many chemopreventive candidate drugs, such as statins, NSAIDs and estrogens, it is reasonable to assume that exposure allocation is unrelated to the outcome of interest, melanoma. In explanation, at the time of prescribing these drugs, both doctors and patients are not aware of potential effects on melanoma incidence. For such research topics, where the presciber is effectively blind for the potential effect of interest, observational research may be as credible as RCTs. [18] Therefore, we

will perform a number of case-control studies in a general population-based dataset linking drug-dispensing data from the pharmaco-morbidity linkage network (PHARMO) with pathological data (PALGA) from the nationwide network and registry of histo- and cytopathology in The Netherlands. By means of this pharmocoepidemiological approach, we will attempt to estimate the causal effects on the incidence and progression of melanoma of a few candidate chemopreventive drugs.

In **chapter 5**, we investigate the association between use of statins and the incidence of CM. In addition, potential effects of prior statins use on Breslow's thickness at diagnosis of CM is studied as well as effects on time to metastasis.
As will be described in chapter 4, non-steroidal anti-inflammatory drugs (NSAIDs), both acetylsaliylic acid (aspirin) and non-acetylsaliylic acid-NSAIDs have been suggested to have beneficial effects on melanoma incidence. Therefore, in **chapter 6**, we will study the association between use of NSAIDs including (low-dose) aspirin on melanoma development.
In **chapter 7** an etiological association study on the association between use of ACE inhibitors and angiotensin receptor antagonists on melanoma incidence and progression is executed.

Gender differences in melanoma have been established on both incidence and prognosis. Interestingly, although melanoma incidence is higher among women, survival is improved in female CM patients as compared to male CM patients. This female survival benefit is maintained after adjusting for well-established prognostic factors. [19] Until now, gender differences in melanoma are not well understood. One of the factors that could play a role in these gender differences are the effects of female hormones, such as estrogens. [20] Therefore, in **chapter 8 and 9**, we will study the association between use of estrogens and development and tumor thickness at diagnosis of melanoma, respectively.

Finally, in **chapter 10** the results of the studies presented in this thesis are interpreted and placed into perspective, the potential of drug chemoprevention for melanoma is discussed, and suggestions for future research are postulated. The theme of this thesis is summarized in **chapter 11**.

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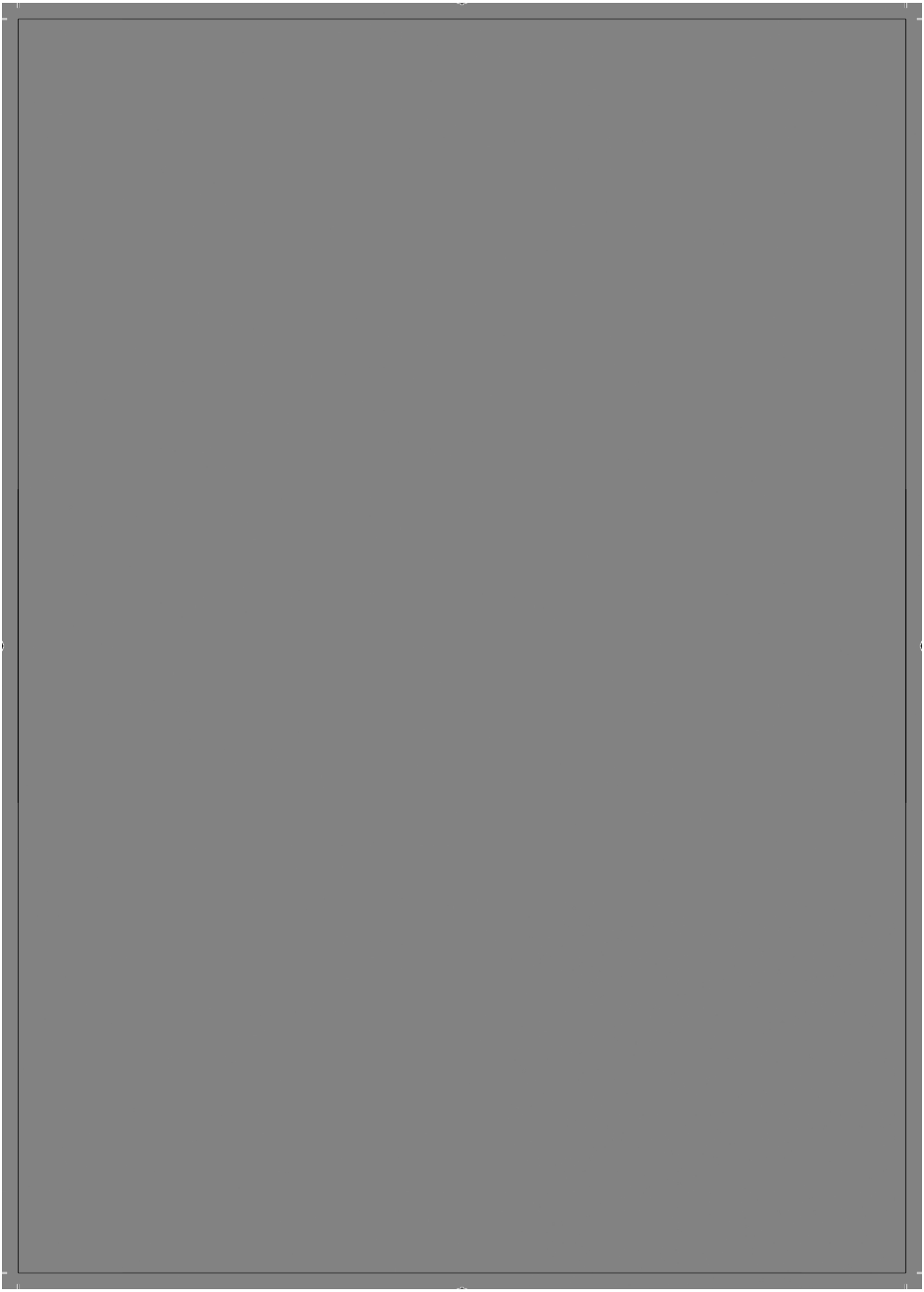

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Chapter 2

Epidemiology of Extracutaneous Melanoma in The Netherlands

Extracutaneous melanoma

Els R. Koomen, Esther de Vries, Leon C. van Kempen, Alexander C.J. van Akkooi, Henk Jan Guchelaar, Marieke J. Louwman, Tamar Nijsten, Jan Willem W. Coebergh

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Abstract

Background: Reliable population-based incidence and survival data on extracutaneous melanoma (ECM) are sparse.

Patients and Methods: Incidence data (1989-2006) from the Netherlands Cancer Registry were combined with vital status on January 1st 2008. Age-adjusted annual incidence rates were calculated by direct standardization and the Estimated Annual Percentage Change was estimated to detect changing trends in incidence. Additionally, we performed cohort-based relative survival analysis.

Results: Ocular melanomas were the most common ECM subsite with European Standardized incidence Rates (ESR) of 10.7 and 8.2 per 1,000,000 person-years for males and females, respectively. In comparison, for cutaneous melanoma (CM), the ESRs for men and women were 122 and 155 per million person-years, respectively.
No statistically significant trends in the incidence of ECM were detected whereas an annual increase of 4.4 percent for men and 3.6 percent for women was detected in the incidence of CM.
Relative survival for ECM was poor, but differed largely between anatomical subtypes ranging from a 5-year relative survival of 74% for ocular melanomas to 15% for certain subsites of mucosal melanomas.

Conclusion: Of all ECM subsites, ocular melanomas had the highest incidence and the best survival. Mucosal melanomas were the second most frequent subsite of ECM. Five-year relative survival for all ECM subtypes was worse if compared to CM. No statistically significant trends in the incidence of (subsites of) ECM were determined.

Impact: This study gives insight into the relative sizes of the different subgroups of ECM as well as an estimate of 5-year survival, which varies substantially by subsite.

Introduction

Although rare, melanomas can arise at noncutaneous sites. Such extracutaneous melanomas (ECM) include ocular, meningeal and mucosal melanomas or melanomas on exceedingly rare sites like the adrenal gland, kidney, lung or soft tissue. Ocular melanomas arise in the eye and adnexa, whereas meningeal melanomas occur in the dura mater or leptomeninges. Mucosal melanomas may occur at different anatomical sites, such as in the head & neck region, female or male genitals, esophagus, anorectally or very rarely in the urinary tract or biliary tract. [1]

Most of the available epidemiological data on ECM is restricted to anatomic sites and not based on well-described populations, e.g., from geographic regions or national databases. [2-5] Thus, population-based incidence and survival data on ECM are sparse. In 2005, McLaughlin *et al.* published incidence data on ECM from the US and showed that ocular melanoma was more common among men (men: 6.8 cases per million, women: 5.3 cases per million women, age-adjusted to U.S. population standard in 2000), whereas mucosal melanomas were more common among women (women: 2.8 cases per million, men: 1.5 cases per million men). [6] Unfortunately, trends in ECM incidence and survival were not reported. Comparable European data are not available.

In general, ECM are rare (incidence rates < 10 per million person years) [2-6] and have a poor prognosis with 5-year survival estimates ranging from 4 to 60 percent [1]. As opposed to cutaneous melanomas (CM), ECM's prognosis is poor due to late diagnosis as most ECM are not visible, early presenting signs and symptoms are often absent. Additionally, ECM seem to be biologically more aggressive than most CM. [1]

In The Netherlands, the age-adjusted incidence rate of CM has increased significantly with 3.3% in men and with 2.2% in women between 1989 and 1998. [7] This is likely due to increases in sun exposure, and partly due to increased awareness. [7] Since effects of sun exposure are considered to be small or absent for the development of ECM, no changes in incidence rates are expected to occur over time for ECM.

The objective of this study was to contribute to the very limited information on population level regarding this rare group of cancers by assessing incidence rates, relative survival and time trends in the incidence of ECM of different anatomical sites in the national general population-based Netherlands Cancer Registry between 1989 and 2006.

Patients and methods

Data

Incidence data from 1989 until 2006 according to sex, calendar year of diagnosis and anatomical site were obtained from the nationwide population-based Cancer Registry in The Netherlands. This registry receives lists of newly diagnosed cases on a regular basis from the PALGA network, the registry of histo- and cytopathology in the Netherlands. All pathology departments in the country participate in this nationwide network. Additional to these records, lists of hospitalized cancer patients are provided by the medical record departments and these are also checked. Sequentially, the medical records of patients with newly diagnosed primaries are collected. From these, trained tumor registrars summarize relevant information. Duplicate records are removed. [8]

From both hospital records and the death registry of the Central Bureau for Genealogy (which registers all deceased in The Netherlands via the municipal civil registries), vital status on January 1st 2008 was obtained. We recorded survival for the time periods between primary melanoma diagnosis and date of death or the latest date of follow-up. Patients who were alive at their last date of follow-up, were considered censored.

Anatomical sites of ECM were identified based on the International Classification of Disease for Oncology, 9th and 10th revision (ICD-9, ICD-10) and regrouped in the melanoma of the CNS (brain, benign brain tumors, meninges and other CNS; ICD codes: 1921-1922, C70-C71), ocular melanoma (eye, eye lids, orbita, choroid, corpus ciliare and the eye muscles; ICD codes: 1900-1909, C69), or mucosal melanoma of the ear, nose & throat region (nasal cavity, middle ear, sinuses, larynx, lip, pharynx and oral cavity; ICD codes: 1404, 1430, 1439, 1452, 1453, 1600-1609, C00-C09, C11-C14, C30-C33), genitals (males: penis and other not otherwise specified male genitals, females: cervix uteri, ovary, vagina and other female genitals, but excluding the vulva; ICD codes: 1840-1848, 1871-1877, C52, C53, C56, C57, C60, C63), vulva (ICD code: C51), gastrointestinal tract (esophagus and anus/anal canal; ICD codes: 1504, 1505, 1541-1548, C15, C20-C21), lung (ICD codes: 1625, C34, C38) or urinary tract (including urinary bladder; ICD codes: 1881, C68), and ECM of other sites (such as adrenal gland, kidney, soft tissue; ICD codes: 1890, C49, C74, C77). ECM of the stomach, small intestine and colorectal are exceedingly rare and can be metastases of an occult primary melanoma. Therefore, ECM registered as the subsites stomach, small intestine and colorectal (ICD codes 1521, 1570, C16, C17 and C18) were excluded from analyses (n=10).

The Netherlands Cancer Registry records are assumed to be complete from 1989 onwards. [9] However, data collection before 2003 on ocular melanomas was incomplete because, at the time, non-pathologically confirmed ocular melanomas were not systematically included in the Netherlands Cancer Registry. Likewise, vulvar melanomas were not systematically reported prior to 1993 because a unique ICD code was lacking. Consequently, we included only data from 2003 and 1993 onwards for ocular and vulvar melanomas, respectively.

Analysis

For each site, incidence rates were calculated stratified by sex and calendar year. Annual incidence rates were age adjusted by direct standardization according to the European Standard Population, resulting in European standardized incidence rates (ESR) per million person-years. Subsequently, 3-year moving averages of the ESR were calculated. To detect changing trends in ECM incidence over time, the Estimated Annual Percentage Change (EAPC) was calculated. The EAPC was estimated by fitting a regression line with the following equation: $y = mx + b$, where y = ln ESR and x = calendar year. The EAPC is then equal to $100*(e^m - 1)$. This method assumes that the incidence rates increase or decrease at a constant rate in the study period (1989-2006). For each EAPC, 95% confidence intervals were calculated using the standard error of m obtained with the regression line. [7] EAPCs were calculated separately for men and women, for CM, all mucosal melanomas, and mucosal melanomas of the vulva and Ear-Nose-Throat region.

Additionally, joinpoint analyses were carried out to determine if significant changes in the time trends were present and, if so, when they occurred. [10] In joinpoint analyses, linear line segments are connected on a log scale to identify changes in the EAPC values over time. [10]

Relative survival was estimated in a cohort-based analysis by dividing the crude survival among cancer patients by the expected survival from the general population-based upon the same age- and sex-distributions as has been described earlier. [11] Relative rather than crude survival was estimated because these reflect the excess mortality among the cancer patients rather than the overall survival experience of the patients, including the non-cancer related deaths. Standard errors were calculated according to Greenwood's method. [12]

All calculated *p*-values were two-sided and considered significant if $p<0.05$. All analyses were performed using SPSS 16.0 (SPSS Inc. Chicago, IL), except relative survival which was calculated using the SAS computer package, version 9.1 (SAS Institute Inc., Cary, NC).

Results

Between 1989 and 2006, a total number of 3134 primary invasive ECM were registered among Dutch citizens aged 18 years or older. In comparison, the Netherlands Cancer Registry recorded 42,124 primary invasive CM in the same period. The number of melanomas with an unknown primary was <0.2% and these were considered to have a cutaneous origin in this study.

Incidence

Table 1 summarizes the number of incident melanoma cases diagnosed between 1989 and 2006 by anatomical location and sex. During this period, a total of 42,124 CM were diagnosed. The age-standardized incidence rates (ESR) of CM were 122 and 155 per million person-years for males and females, respectively (Table 1). The male-to-female rate ratio was 0.79.

Between 2003 and 2006, ECM compromised 6.4% of all invasive melanomas. The proportion of ECM was slightly higher among men (7.0% versus 6.0%).

During this period, ocular melanomas were the most commonly occurring subsite of ECM and represented 87% and 68% of all ECM among men and women, respectively. The ESRs of ocular melanoma were 10.7 and 8.2 per 1,000,000 person-years for males and females, respectively. Thus, the male-to-female rate ratio of ocular melanomas was 1.3.

After excluding ocular melanomas reported before 2003 and vulvar melanomas reported before 1993 (see method section for explanation), 1502 incident primary ECM among 1493 patients were eligible for further analyses.

Patients with ECM had a median age at diagnosis of 68 years whereas CM patients had a median age of 53 years. Median ages at diagnosis and the 25th and 75th percentile of patients with different melanoma subtypes are presented in Table 1. Overall, ECM patients are generally older at diagnosis than CM patients and male ECM patients are younger at diagnosis (median age: 65 years) than female ECM patients (median age: 71 years).

Mucosal melanomas, such as vulvar (ESR 1.06) and ECM of the ear, nose and throat (ESR 0.88 for males and 0.71 for females) also contributed substantially to the total ECM incidence. The male-to-female rate ratio of mucosal melanomas was 0.48.

Only 13 incident primary ECM within the central nervous system were reported in the total study period (1989-2006) resulting in extreme low ESRs for men and women (0.038 and 0.052 per million person-years, respectively).

Table 1 Invasive Cutaneous and Extracutaneous Melanomas in the Netherlands National Cancer Registry

Anatomical location	Men		Women		Both Sexes	
	Incident cases (n)	ESR 1989 - 2006 (rate [1])	Incident cases (n)	ESR 1989 - 2006 (rate [1])	5y-Relative Survival [2] (%)	Median age (years) [3]
Skin	17,723	121.9	24,401	155.2	86 (86-87)	53 (40-66)
Non Skin, Non-Mucosal						
CNS [4]	6	0.038	7	0.052	-	51 (32-60)
Ocular [4,5]	373	10.67	322	8.19	74 (67-81)	62 (54-72)
Others [4]	1	0.01	4	0.03	-	61 (51-74)
Non Skin, Mucosal						
Ear-Nose-Throat [4]	122	0.880	139	0.708	27 (20-34)	71 (60-80)
Genitals [4]	48	0.338	121	0.653	26 (18-34)	72 (58-81)
Vulva	n.a.	n.a.	214	1.06	40 (31-49)	75 (65-83)
Gastrointestinal tract [4]	53	0.382	78	0.400	15 (8-22)	72 (59-80)
Lung [4]	6	0.045	1	0.009	-	66 (59-79)
Urinary tract [4]	1	0.007	6	0.031	-	71 (67-82)

[1] ESR = European Standarized Incidence Rate, expressed in 1 per 1.000.000 person years.
[2] Calculated 5-year cumulative overall survival relative to the general Dutch population standarized for age and gender.
[3] Median age at diagnosis In years and 25 and 75 percentile.
[4] The extracutaneous localizations were defined as:
- Central Nervous System (CNS) includes brain, benign brain tunours, meninges and other CNS.
- Ocular includes melanoma of the eye and its adnexa, such as the eye lids, orbita, choroidia, corpus ciliare and the eye muscles.
- Others includes adrenal gland, kidney and soft tissue.
- Mucosal melanomas were subdivided in several categories, such as:
- Ear-Nose-Throat which included sinonasal and oropharyngeal mucosal melanomas (larynx, lip, pharynx, oral cavity, nasal cavity, middle ear and sinuses).
- Genitals which included for males: penis and other NOS (not otherwise specified) male genitals and for women: female genitals including cervix uteri, ovary, vagina and other female genitals, but excluding the vulva.
- Gastrointestinal tract which included oesophagus and anus/anal canal.
- Urinary tract which included urinary bladder and other urinary tract structures.

[5] Only data from 2003 intil 2006 were included for ocular melanomas since the Dutch Cancer Registry was incomplete for ocular melanomas before 2003.

Relative survival

Five-year relative survival for CM unstratified for gender was 86% overall between 1989 and 2006. Relative survival of all ECM subtypes was poor compared to those of CM. However, there are large differences in 5-year-relative survival estimates between ECM subtypes. Of all ECM, ocular melanomas had the best 5-year relative survival of 74% whereas vulvar melanomas had a 5-year relative survival of 40%. The 5-year relative survival of different subsites of mucosal melanomas varied between 15% and 40% (Table 1).

Trends in incidence

For both sexes, the ESR for CM increased significantly between 1989 and 2006 (Table 2). For males, the ESR for CM increased with 4.4% (95%CI: 3.9, 4.9%) per year. Increases among females were 3.6% (95%CI: 2.9, 4.2%).

The age-adjusted incidence rates of all mucosal melanomas and of the selected mucosal region of ear-nose-throat (Fig. 1) showed an increasing, but nonsignificant trend among women (EAPC: 1.8%, 95%CI: –0.5, 4.2%, and EAPC: 2.8%, 95%CI: –0.1, 6.8%, respectively). For men, lower increases were observed in the annual incidence of all mucosal melanomas and these of the ear, nose and throat region (EAPC: 1.0%, 95%CI: –1.8, 3.8, and EAPC: 1.1%, 95%CI: –4.4, 7.1, respectively). The estimated increase in incidence of vulvar melanoma between 1993 and 2006 was only 0.3% (95% CI: –2.6, 3.4).

Despite apparent changes in trend, no statistically significant joinpoints were demonstrated in the joinpoint analyses that were carried out (results not shown).

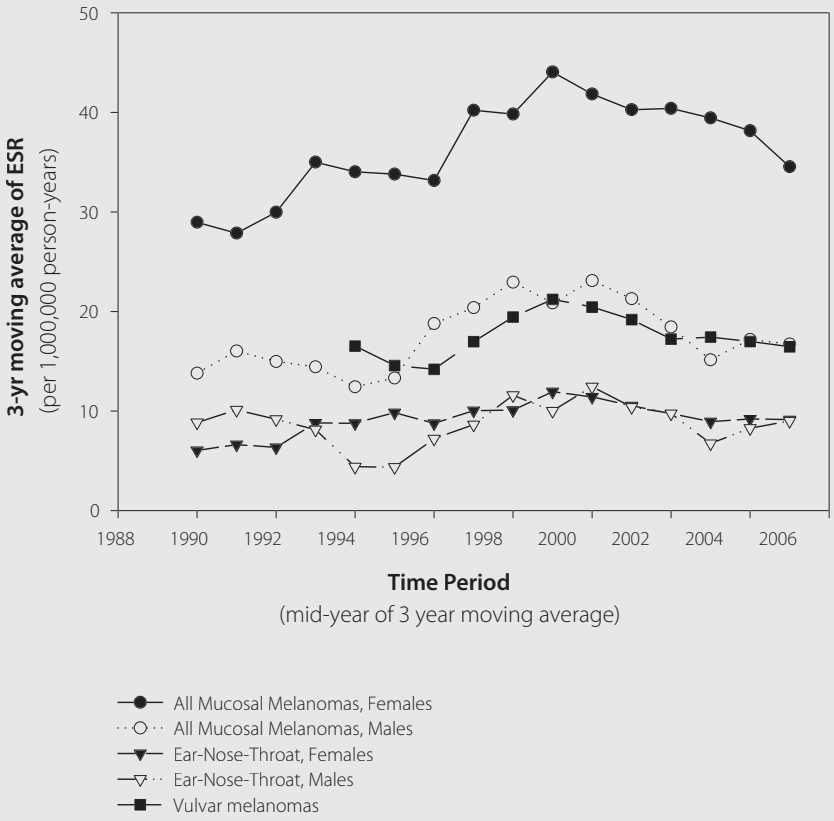
Table 2 European Standardized Incidence Rates for melanomas of different locations in 3-year periods between 1989 and 2006

	Time period												EAPC [2]	
Gender & location	1989-1991		1992-1994		1995-1997		1998-2000		2001-2003		2004-2006			
	n	ESR[1]	n	ESR[1]	n	ESR[1]	n	ESR[1]	n	ESR[1]	n	ESR[1]	mean	95% CI
Men														
Skin	1,900	88.5	2,154	96.0	2,600	111.6	3,007	124.1	3,605	142.6	4,457	168.9	**+4.4%**	**+3.9 to +4.9%**
Mucosal [3]	28	1.95	29	2.54	38	1.97	47	3.21	41	1.67	43	3.14	+0.9%	-2.0 to +3.9%
Ear-Nose-Throat [3]	18	0.88	17	0.81	16	0.72	23	1.00	24	0.97	24	0.90	+1.1%	-4.4 to +7.1%
Ocular [3]	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	289	10.92	n.a.	n.a.
Women														
Skin	2,909	124.7	3,149	130.8	3,699	150.8	4,063	161.2	4,892	189.5	5,689	213.6	**+3.6%**	**+2.9 to +4.2%**
Mucosal [3]	71	2.89	87	3.65	83	2.58	111	3.21	104	2.41	104	2.78	+1.8%	-0.5 to +4.2%
Ear-Nose-Throat [3]	15	0.60	22	0.88	22	0.87	30	1.19	25	0.97	25	0.91	+2.8%	-0.1 to +6.8%
Vulva [4]	n.a.	n.a.	n.a.	n.a.	35	1.42	55	2.12	45	1.72	47	1.64	+0.3%	-2.6 to +3.4%
Ocular [3]	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	234	7.94	n.a.	n.a.

[1] European Standarized Incidence Rate, expressed in 1 per 1.000.000 person years.
[2] EAPC = Estimated Annual Percentage Change, data printed in bold if statistically significant ($p<0.05$).
[3] Subcategories of the extracutaneous melanomas that were tested for an incidence trend were:
- Mucosal melanomas includes all mucosal melanomas (Ear-Nose-Throat, Genitals, Vulva, Gastrointestinal tract, Lungs and Urinary tract).
- Ear-Nose-Throat which included only the sinonasal and oropharyngeal mucosal melanomas (larynx, lip, pharynx, oral cavity, nasal cavity, middle ear and sinuses).
- Vulvar mucosal melanomas.

[4] Before 1993 there were no separate ICD codes registered for vulvar melanomas. Both in 1993 and 1994, 16 vulvar melanomas were registered.

Figure 1 Trends in the incidence of muscosal melanomas in The Netherlands: 1989 - 2006



Discussion

Incidence

Our results show that, between 2003 and 2006, about 6.4% of all primary melanomas in The Netherlands were ECM. This proportion is similar to previous reports (4-6.8%). [6,13] In general, ECM patients, especially those with mucosal melanomas, are older at diagnosis than CM patients. Similarly, Chang *et al.* observed a median age of ~ 70 years for mucosal melanomas. [15]

No statistically significant time trend in the ECM incidence was observed, whereas an annual increase in age-adjusted standardized CM incidence among both sexes was observed.

Ocular melanoma was the most common ECM subsite and its' incidence was somewhat higher than reported by McLaughlin *et al.* (ESR females: 10.7 versus 6.8 per million person-years; males: 8.2 versus 5.3 per million person-years) [6]. The male-to-female rate ratio of 1.3, however, was similar [6].

Mucosal melanomas were the second most frequent subsite of ECM and the incidence we report is in agreement with the US data reported in McLaughlin's paper (ESR men: 1.8 versus 1.5 per million person-years; women: 2.8 versus 2.8 per million person-years) [6]. The male-to-female rate ratio for mucosal melanomas was 0.48 which seems to be rather consistent throughout the literature [6,14,15], and the female predominance is most likely a reflection of the lack of a male counterpart for vulvovaginal lesions. [14]

The incidence of vulvar melanoma in our study (ESR: 1.1 per million person-years) is similar to a previously published study from Sweden (1960-1984) [3]. In their study, however, the annual age-standardized incidence of vulvar melanoma decreased with 3.2% annually (mainly due to a decrease among younger age groups) [3], whereas our results showed no definite trend in incidence (EAPC 0.3%, 95% CI: –2.6 to +3.4%). These Swedish data are, however, outdated (data up to 1984) and were based on a consecutive series of cases rather than a population-based sample. [3]

Relative survival

Five-year relative survival proportions of ECM subtypes, except ocular melanoma, were poor compared to CM (86%) and differed substantially between subsites. Preferably, we would have stratified for the clinical stage of disease at diagnosis in the survival analysis which was not possible due to low numbers of incident ECM.

Of all ECM subsites, patients with primary ocular melanoma had the best survival with a relative 5-year survival of 74% (95% CI: 67-81%). Estimates from the Collaborative Ocular Melanoma Study (COMS) [1] were slightly lower (60%), but the 5-year disease specific survival of 75 percent published by Chang *et al.* [15] is comparable. Our estimate may be somewhat underestimated due to the fact that we could only use data from 2003 until 2006 and vital status on the 1st of January 2008 resulting in relatively short follow-up for part of the patients with ocular melanoma in our dataset and hence relatively many patients being censored alive.

Vulvar melanomas in our dataset resulted in a 5-year survival proportion of 40% (95% CI: 31-49%), comparable with the 50% reported by Weinstock on US data [17] .

The survival proportion for gastrointestinal melanoma was calculated to be 15%

(95%CI: 8-22%), slightly better than the overall crude 5-year survival of 6% presented in a Dutch case series of anorectal melanoma (63 cases, 1960-1995) [16]. Although we included anorectal as well as esophageal melanomas in this subsite, survival estimates for patients with anorectal and esophageal melanomas did not substantially differ in our dataset (data not shown).

Reflection

The poor survival proportions estimated for ECM could obviously reflect the often advanced stages in which ECM are diagnosed. However, ECM and CM also differ substantially in their clinicopathologic and molecular aspects. Even between subgroups of CM, such as acral melanoma and chronic versus non-chronic sun exposed melanomas, the genetic makeup and morphological features differ. [18] The clinical heterogeneity of melanoma can, in part, be explained by distinct sets of genetic alterations. Approximately 80% of melanomas in skin without chronic sun-induced damage contain a mutation in either BRAF or NRAS, whereas cutaneous melanoma arising in non-damaged skin, as well as acral and mucosal melanomas do not. [19] Instead, these tumors frequently display increased gene copy number of cyclin-dependent kinase 4 and cyclin D1. Oncogenic BRAF mutations in ocular melanoma are rare, if not absent, or restricted to only a subset of cells in posterior uveal melanomas. [20-23] However, somatic mutations in the heterotrimeric G protein alpha-subunit, GNAQ, are frequently observed in uveal melanoma, but rarely in other melanomas. [24] Mutations and/or copy number increases of receptor tyrosine kinase KIT have been detected in 39% of mucosal, 36% of acral, and 28% of melanomas on chronically sun-damaged skin. [25] These genetic changes commonly result in various alternative routes to MAP kinase activation and hence proliferation. However, upstream oncogenic mutations in BRAF, NRAS, KIT, and GNAQ will activate additional signaling cascades specific for that tumor type and therefore contribute to the diversity in melanoma biology, prognosis, and response to therapy.

Future research

Future epidemiological research on ECM should include large (international) datasets. This would enable researchers to stratify for clinical stage at diagnosis in survival analysis and therefore to study how much of the poor prognosis of ECM is due to delayed diagnosis. It would also allow for studying the male-to-female ratios reported and time trends in incidence and survival, investigate possible geographical gradients in comparison with CM. Ideally, these datasets would be population-based to avoid biases occurring from selected patient groups. Whenever possible such international

databases should include aspects that may explain the clinical heterogeneity, such as the morphological features and mutational status of an ECM. If treatment were to be adequately collected, the effects of targeted therapies such as imatinib for c-kit mutated mucosal melanomas could be studied.

Conclusion

With incidence rates for different subsites of extracutaneous melanoma ranging from less than 0.1 per million person-years for ECM of the lung or urinary tract until about 10-11 per million person-years for ocular melanomas among men, ECM is a rare type of melanoma. Of all ECM subsites, ocular melanomas had the highest incidence (10.7 and 8.2 per million person-years for men and women, respectively) and the best survival with a 5-year relative survival of 74%. Mucosal melanomas, such as vulvar melanomas, were the second most frequent subsite of ECM. Five-year relative survival for mucosal melanomas ranged between 15 and 40% and survival for all ECM subtypes was worse if compared to the 86% five-year relative survival for CM. Also in contrast with CM, no statistically significant trends in the incidence of (subsites of) ECM were determined.

Acknowledgement

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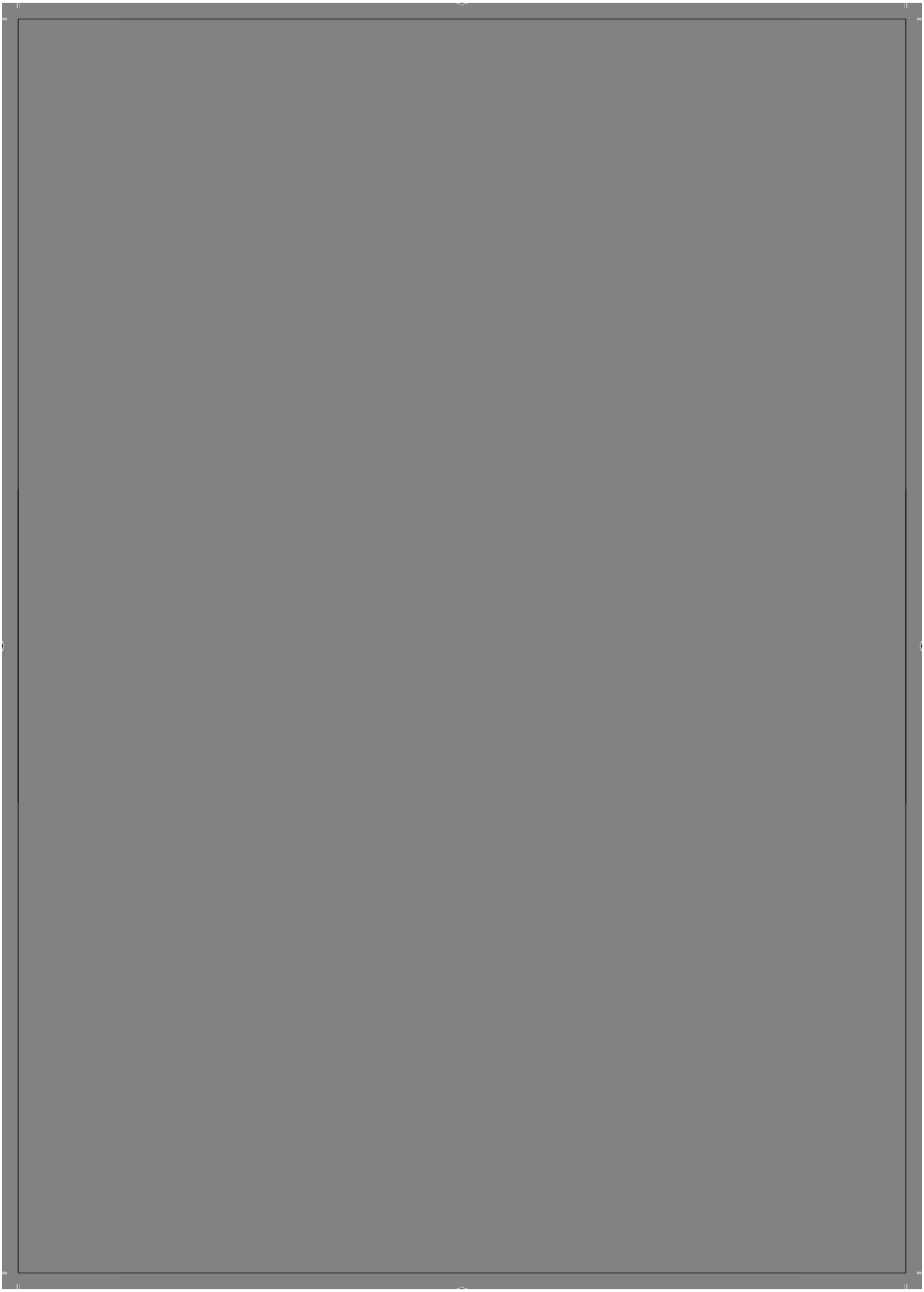

Chapter 3

Burden of disease in Dutch melanoma patients, 1989-2006

3

Burden of melanoma

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Abstract

Background: Burden of disease is a concept describing loss of health and death due to diseases and has not been adequately studied for melanoma.

Patients and Methods: Age- and gender-specific incidence data from all patients diagnosed with melanoma between 1989 and 2006 were obtained from the Netherlands Cancer Registry. Mortality numbers were extracted from the Statistics Netherlands database. Life tables with the probability of developing a melanoma were calculated per 5-year period with use of the DevCan software. The standard life expectancy for both men and women per 5-year age group were estimated using DISMOD software. The Years Lost due to Disability (YLD) and Years of Life Lost (YLL) due to melanoma were calculated using these life tables and life expectancies. The disability adjusted life years (DALY), a general measure for the burden of a disease, was estimated by adding YLD and YLL.

Results: The incidence of melanoma almost doubled between 1989 and 2006 (cumulative incidence rate increased from 1.03-1.31% to 2.02-2.11%). The burden of melanoma to society increased rapidly between 1989 and 2006. On average, patients lived 21.6-28.2 years with a melanoma diagnosis. Melanoma resulted in a loss of 17.8-20.1 years per before the age of 95, for those that died of their melanoma.

Conclusion: Melanoma is becoming a great burden to Dutch society. Health care providers may have to adjust their current policy in treating patients with melanoma.

Abbreviations

YLD Years Lived with Disability; the number of incident cases times the disability weight (0.05) times the average duration of the case until remission or death

AYLD Average Years Lived with Disability; the YLD divided by the number of incident cases

YLL Years of Life Lost; the number of deaths times the standard life expectancy at age of death

AYLL Average Years of Life Lost; the YLL divided by the number of deaths

YLWD Years Lived with Disease; the number of incident cases times the average duration of the case until remission or death

Introduction

In the past three decades the incidence of melanoma has markedly increased in people of European ancestry. In 2005, melanoma was the 8th most common cancer in males and the 5th most common cancer in females in The Netherlands (a total of 3515 cases among 16.4 million inhabitants) (www.ikcnet.nl). De Vries *et al.* have predicted that by 2015 the number of new cases per year will exceed 4800. [1] Compared with most other malignancies, melanoma affects patients at a younger age and has relatively good survival rates for the majority of patients, which have improved over time due to early detection. [2-5] This implies an increasing number of melanoma survivors who live with a cancer diagnosis and it's social and psychological effects and may utilize health care for medical and psychological reasons related to their melanoma history over a prolonged period of time, which can become a great burden for health care providers.

Usually, the magnitude of a cancer problem is expressed in incidence and mortality rates and numbers. However, the magnitude of the societal problem can also be expressed in a quite different way using Burden of Disease concepts that measure the disease burden for individuals or populations. These burden of disease measures may be used for research purposes, public health campaigns and for the allocation of limited health care resources. The burden of a disease can be estimated by calculating the number of years of life lost (YLL), the number of years of life lived with disease (YLD) and Disability Adjusted Life Years (DALY). [6] These additional measures are of

key importance in estimating the burden of cancer types that occur in young patients and often have a favorable prognosis.
Only a few studies have investigated the burden of melanoma. Brochez and colleagues investigated the burden of melanoma in Belgium, expressed as years of potential life lost and showed that in those terms, melanoma was the second most important cancer of all adult-onset cancers. [7] Melanoma resulted in a loss of 8 years before the age of 65 in males and 6 years in females. In the United States, the burden of melanoma has also been expressed by years of potential life lost and these rates were one of the highest for adult-onset cancers. [8] None of these studies evaluated changes in the burden over time, nor did they include the part of the population aged over 65, which is continuously growing in many European countries and therefore represents a population group which is of increasing importance.
In the Netherlands, the burden of melanoma has never been estimated by YLL, AYLL, YLD or DALYs. Therefore, we estimated the size of the burden of melanoma within the general Dutch society with these four measures using data for 1989-2006 in 4 time periods (1989-1991, 1992-1996, 1997-2001, and 2002-2006).

Patients and methods

Population

Age- and gender-specific data on newly diagnosed patients with melanoma (ICD-0 codes: C44.0-C44.9) were obtained from the Netherlands Cancer Registry, which collects incidence and tumor data on all newly diagnosed cancers in the Netherlands from the regional comprehensive cancer centers since 1989 (i.e., only first melanoma's were used for this study). We used incidence data for 1989 to 2006. Annual data on age and gender of cancer fatalities and population composition were obtained from Statistics Netherlands.

Study design

To estimate the burden of melanoma, we calculated Disability Adjusted Life Years (DALYs) by adding the number of Years of Life Lost (abbreviated YLL) by a person as a consequence of premature death due to melanoma plus the number of years of lived with disability (abbreviated YLD) caused by melanoma by a person . According to Murray *et al.*, one DALY represents the loss of one year of life lived in full health. The sum of these DALYs across the population, or the burden of disease, can be thought of as "a measure of the gap between the current health status and an ideal health

situation in which the entire population lives to an advanced age, free of disease and disability". [9]

Statistical methods

All analyses were performed for 5-year periods (except for period 1989-1991, as data was only available for 18 years) and stratified for gender. The cumulative incidence was calculated per 5-year age group by dividing the number of patients with melanoma by the total population without melanoma and totaling these results. European standardized incidence rates (ESR) were then calculated by multiplying the incidence rates with standard European population data (http://seer.cancer.gov/stdpopulations/). To calculate the probability of a person being newly diagnosed with a melanoma during the 5-year period we used the life table method, which unlike cumulative incidence data, takes into account that the cause of death of a melanoma patient might not be related to melanoma. Also, this method calculates the probability of being diagnosed with melanoma and dying from it, for people without a history of melanoma. The DevCan software program, which was developed by the National Cancer Institute in the United States, was used to calculate these probabilities. [10] For these calculations the following assumptions were made:

(a) The incidence of melanoma is constant in each 5-year period;

(b) The probability of death not being caused by melanoma is the same for melanoma patients as for people without a history of melanoma;

(c) The data obtained from the Netherlands Cancer Registry and Statistics Netherlands were for 5-year age groups. To raise the accuracy, DevCan divides these age groups into 10 periods of 6 months. In each 6 month age group the incidence and mortality rates increase in 10 equal steps and are constant in each 6 month age group. This leads to an exponential decrease with age in each 6-month age group. The numbers of patients at risk and the probability of being diagnosed with a melanoma can therefore be more accurately calculated;

(d) All melanoma specific mortalities are registered with the Netherlands Cancer Registry.

To estimate YLD, we multiplied the number of incident cases by the average duration a patient lives with melanoma in The Netherlands and a weighing factor, determined by the World Health Organization (WHO), that reflects the impact of melanoma on health related quality of life on a scale from 0 (perfect health) to 1 (dead). Melanoma disease duration was estimated using DISMOD. [11] YLLs were calculated using the appropriate life tables. YLL corresponds to the number of deaths due to melanoma

multiplied by the standard life expectancy in the general population at the age which death occurs as estimated by a standard life table. [6] The average years of life lost (AYLL) were calculated by dividing the YLL by the number of melanoma deaths. DALYs were calculated as the sum of the YLL due to premature mortality in the population and the YLD for incident cases of the health condition (i.e., melanoma). To calculate the actual years a patient lives with their melanoma, the years lived with disease were calculated (YLWD). Therefore, we multiplied the number of melanoma patients with their life expectancy at time of diagnosis.

Results

Incidence and mortality

Between 1989 and 1991, an average of 1603 Dutch people were newly diagnosed with melanoma per year (Table 1A and 1B); this increased to 3171 individuals per year in the period 2002-2006. Of all newly diagnosed melanoma patients, 43.3% was male (ESR 15.9 per 100,000 person-years) and 56.7% was female (ESR 19.5 per 100 000 person-years) (Table 1). Cumulative incidence rates almost doubled in men (1.03% in 1989-1991 to 2.02% in 2002-2006) and increased from 1.31% to 2.13% in the same time period for females.

Age at diagnosis of melanoma increased over time; patients diagnosed in 1989-1991 were predominantly diagnosed at an age of 35-50 years (both males and females) whereas people newly diagnosed with melanoma between 2002-2006 were often older (men: mainly 55-70 years, women: mainly 40-60 years) (Fig. 1).

Mortality slowly increased from 182 to 333 males and 182 to 257 females by 2002-2006. Cumulative mortality rates also doubled up to 0.61 for males and up to 0.40% for females. An increase of melanoma mortality was particularly observed for men aged 55 to 65 and females >75 years.

Probability of being diagnosed with melanoma and to die from it

DevCan produced estimations of the probability for a person to develop a melanoma and the probability of dying from a melanoma in a certain age group (Table 2). In 2006, male newborns had an overall chance of 1 in 62 to develop a melanoma, for female newborn this was 1 in 50. A man of 40 years old had a probability of 1.1% to develop a melanoma before the age of 75 years. For females, this probability was 1.2%. Men were more likely to die of a melanoma; the probability for a 40-year old male to die due to melanoma before the age of 75 was 0.3%. By the age of 65, this

Table 1 Incidence, mortality and burden of disease of Dutch melanoma patients

	Males				Females			
	1989-1991	1992-1996	1997-2001	2002-2006	1989-1991	1992-1996	1997-2001	2002-2006
Number of new melanoma patients	1900	3810	5154	6859	2909	5508	6986	8998
Age standardized incidence rate	8.87	10.08	12.83	15.87	12.25	13.27	15.93	19.53
Cumulative incidence risk	1.02	1.22	1.60	2.00	1.31	1.40	1.71	2.11
Cumulative incidence rate	1.03	1.23	1.61	2.02	1.31	1.41	1.73	2.13
Number of melanoma deaths	546	1072	1335	1664	547	965	1068	1293
Age standardized mortality rate	2.62	2.90	3.37	3.84	2.21	2.20	2.22	2.48
Cumulative mortality risk	0.37	0.44	0.53	0.60	0.30	0.31	0.33	0.39
Cumulative mortality rate	0.37	0.44	0.53	0.60	0.30	0.31	0.33	0.39
YLL	47	51	61	74	54	53	54	63
YLD	10	11	14	18	19	20	25	31
DALYs	57	63	75	92	73	74	79	94
YLWD	199	224	284	368	373	408	495	617
AYLD	1.2	1.1	1.1	1.1	1.5	1.4	1.4	1.4
AYLWD	23.2	22.4	21.6	21.6	29.1	28.8	28.3	28.2
AYLL	19.3	18.2	17.9	17.8	22.4	21.5	20.3	20.1

All numbers except for the number of new melanoma patients are expressed per 100 000 Dutch inhabitants
YLD: years lived with disability; YLWD: years lived with disease; YLL: years of life lost; DALY: disability adjusted life years; AYLD: average years lived with disability; AYLWD: average years lived with disease; AYLL: average years of life lost.

Figure 1 European standardized incidence rates by age at diagnosis

A: men
B: women

Table 2 Percentage of men and women who develop melanoma by a specific age (Z), given cancer free at current age (Y), 2006

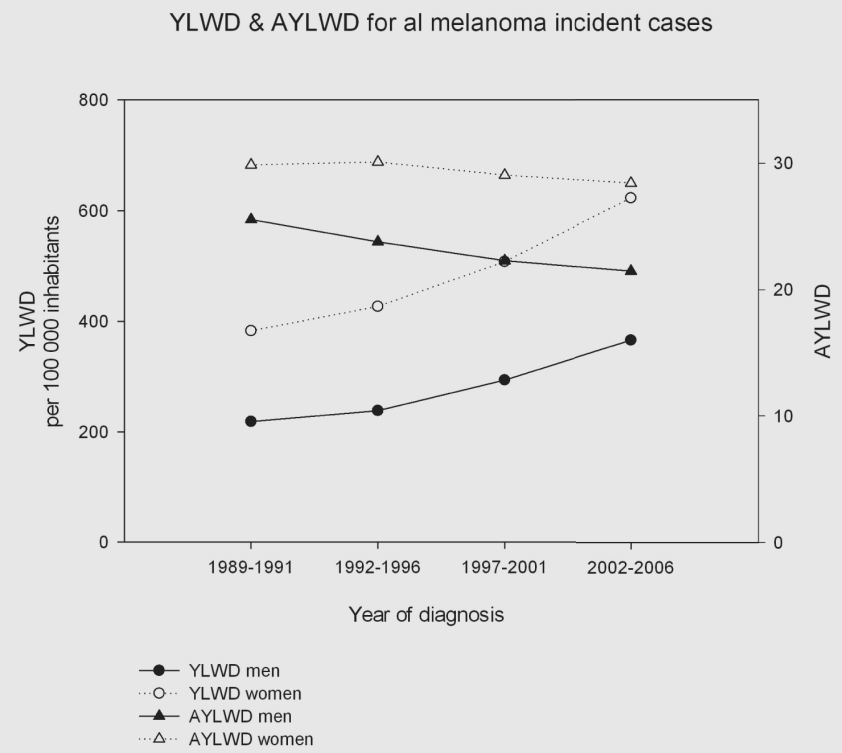
Men

	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	95+
0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.3	0.4	0.5	0.7	0.9	1.1	1.3	1.4	1.5	1.6	1.6	1.6
5		0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.3	0.4	0.5	0.7	0.9	1.1	1.3	1.4	1.5	1.6	1.6	1.6
10			0.0	0.0	0.0	0.1	0.1	0.2	0.3	0.4	0.5	0.7	0.9	1.1	1.3	1.4	1.5	1.6	1.6	1.6
15				0.0	0.0	0.1	0.1	0.2	0.3	0.4	0.5	0.7	0.9	1.1	1.3	1.4	1.5	1.6	1.6	1.6
20					0.0	0.1	0.1	0.2	0.3	0.4	0.5	0.7	0.9	1.1	1.3	1.4	1.5	1.6	1.6	1.6
25						0.0	0.1	0.2	0.3	0.4	0.5	0.7	0.9	1.1	1.2	1.4	1.5	1.5	1.6	1.6
30							0.1	0.1	0.2	0.3	0.5	0.6	0.8	1.0	1.2	1.4	1.5	1.5	1.5	1.5
35								0.1	0.2	0.3	0.4	0.6	0.8	1.0	1.2	1.3	1.4	1.5	1.5	1.5
40									0.1	0.2	0.3	0.5	0.7	0.9	1.1	1.2	1.3	1.4	1.4	1.4
45										0.1	0.2	0.4	0.6	0.8	1.0	1.1	1.3	1.3	1.3	1.3
50											0.1	0.3	0.5	0.7	0.9	1.1	1.2	1.2	1.2	1.2
55												0.2	0.4	0.6	0.8	0.9	1.1	1.1	1.1	1.1
60													0.2	0.5	0.6	0.8	0.9	1.0	1.0	1.0
65														0.2	0.4	0.6	0.7	0.8	0.8	0.8
70															0.2	0.4	0.5	0.6	0.6	0.6
75																0.2	0.4	0.5	0.5	0.5
80																	0.2	0.3	0.3	0.4
85																		0.2	0.2	0.2
90																			0.1	0.2
95																				0.3

Women

	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	95+
0	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.3	0.5	0.7	0.8	1.0	1.2	1.3	1.5	1.7	1.8	1.9	2.0	2.0
5		0.0	0.0	0.0	0.1	0.1	0.2	0.3	0.5	0.7	0.8	1.0	1.2	1.3	1.5	1.7	1.8	1.9	2.0	2.0
10			0.0	0.0	0.1	0.1	0.2	0.3	0.5	0.7	0.8	1.0	1.2	1.3	1.5	1.7	1.8	1.9	2.0	2.0
15				0.0	0.1	0.1	0.2	0.3	0.5	0.7	0.8	1.0	1.2	1.3	1.5	1.7	1.8	1.9	2.0	2.0
20					0.0	0.1	0.2	0.3	0.5	0.6	0.8	1.0	1.2	1.3	1.5	1.7	1.8	1.9	2.0	2.0
25						0.1	0.2	0.3	0.4	0.6	0.8	0.9	1.1	1.3	1.5	1.7	1.8	1.9	1.9	1.9
30							0.1	0.2	0.4	0.5	0.7	0.9	1.1	1.2	1.4	1.6	1.7	1.8	1.9	1.9
35								0.1	0.3	0.4	0.6	0.8	1.0	1.1	1.3	1.5	1.6	1.7	1.8	1.8
40									0.1	0.3	0.5	0.7	0.8	1.0	1.2	1.4	1.5	1.6	1.7	1.7
45										0.2	0.3	0.5	0.7	0.9	1.1	1.3	1.4	1.5	1.5	1.5
50											0.2	0.3	0.5	0.7	0.9	1.1	1.2	1.3	1.4	1.4
55												0.2	0.4	0.6	0.8	1.0	1.1	1.2	1.2	1.2
60													0.2	0.4	0.6	0.8	0.9	1.0	1.1	1.1
65														0.2	0.4	0.6	0.8	0.8	0.9	0.9
70															0.2	0.5	0.6	0.7	0.7	0.7
75																0.2	0.4	0.5	0.6	0.6
80																	0.2	0.3	0.4	0.4
85																		0.2	0.3	0.3
90																			0.2	0.2
95																				0.1

Figure 2 Years Lived with Disease (YLWD) and Average Years Lived with Disease (AYLWD) by year of diagnosis



probability had decreased to 0.1%. Corresponding probabilities for females were 0.2% for a woman aged 40 and 0.1% for women aged 65.

Years Lived with Disability (YLD)

The average number of years that a male melanoma patient lived with melanoma, adjusted for disability due to melanoma (disability weight: 0.05) decreased from 1.16 years in 1989-1991 to 1.08 years in 2002-2006. Females had a higher AYLD: 1.46 years in 1989-1991 and 1.41 years in 2002-2006 compared to men (Table 1).

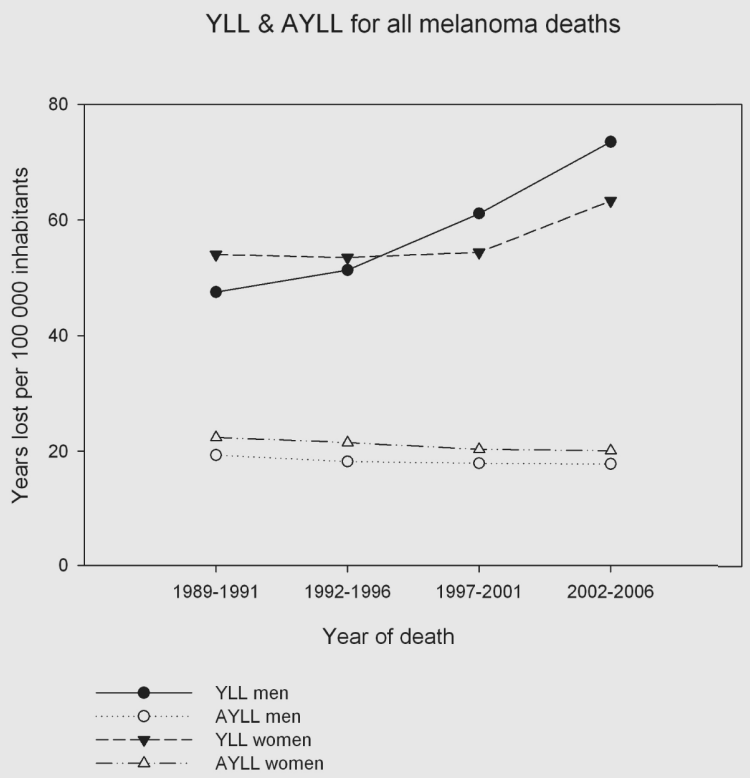
In contrast to the slight decreases in AYLD, the total YLD of melanoma in the general population rapidly increased for both sexes. For men, the YLD increased from 10 to 18

years per 100 000 inhabitants (1989 to 2006) and from 19 (1989-1991) to 31 years (2002-2006) for women.

Years lived with disease (YLWD)

The Average Years Lived with Disease (AYLWD), without adjustments for disability, decreased for both sexes, from 23.2 to 21.6 years for men and 29.1 to 28.2 years for females (Table 1 and Fig. 2). The total years of life with melanoma in the general population has rapidly increased. For men a total of 365.8 life-years lived with melanoma per 100 000 inhabitants in 2002-2006 was estimated compared to 198.7 years in 1989-1991. For women the YLWD rose from 373.3 to 616.9 (Fig. 2).

Figure 3 Years of live lost (YLL) and Average Years of Life Lost (AYLL) by years of diagnosis



Years of life lost (YLL)

In 1989-1991, a male melanoma patient lost on average 19.3 life-years (AYLL) which decreased to 17.8 years in 2002-2006. For females the AYLL also decreased from 22.4 to 20.1 years. However, the total YLL to melanoma in the Dutch population almost tripled for men and more than doubled for females. In 2002-2006 the total YLL for melanoma for females was 63 years per 100 000 inhabitants (Table 1). Analyses of YLL per 5-year age group showed that the YLL of men aged 50-65 years increased most notably over 1989 to 2006. For women the YLL increased especially for women aged 50 to 80 years and aged 35-39 (data not shown).

Disability Adjusted Life Years (DALY)

The burden of melanoma as estimated by DALYs per 100 000 inhabitants also increased over 1989-2006 (men: 57 to 92 and females: 73 to 94 inhabitants). The increase over 1989-2006 was steeper for men than women, but the increase of the DALYs appeared comparable for both sexes in the 2002-2006.

Discussion

The high YLD and YLLs for melanoma patients emphasize the impact of melanoma on (specialized) health care and the increasing melanoma incidence suggests that this will further rise in the future. YLD and YLWD emphasize the importance of burden-of-disease-concept measures as they estimate the number of years patients might be in need for additional (psychological) care. This is in contrast to incidence rates, which only mark the increasing number of patients that will require treatment and follow-up. The high estimates of the burden of disease concepts also illustrate that there is profit to be gained in the management of melanoma patients and its survivors.

Increases in mortality of melanoma in the Netherlands were modest and much smaller than those observed for incidence; the burden of melanoma in terms of YLL the Netherlands increased considerably between 1989 and 2006 up to an YLL of 20. This high YLL is due to the fact that many patients are middle aged when diagnosed with their first melanoma and that most of those who die of melanoma die fairly soon after the diagnosis. Our results cannot be directly compared to the few other studies looking at burden of disease measure for melanoma, as the other studies used a cut-off value of 65 years. [7, 8] Although a cut-off of value of 65-years to calculate YLL is commonly used for the determination of premature mortality in an occupational

population to assess loss of productivity, analyses unrestricted by age are needed to assess the duration that people are affected by a disease. For this reason, we decided not to use this age cut-off. Moreover, most melanoma patients are diagnosed at an age of 55-65 years and most patients have a 5-10 year survival rate of >90%. [2] Dutch melanoma patients live to be on average 75 years of age, a cut-off value of 65 years would underestimate the YLL with about 10 years.
AYLL is calculated as YLL divided by the number of melanoma deaths. However, if you include all melanoma patients (dead or alive) and not just those who died of melanoma, melanoma was associated with a mean loss of approximately three years of life for an individual melanoma patient between 2002 and 2006. Although the numbers of life years lost per patient and life years lived with disease are slightly decreasing over time, reflecting improving survival and a slightly increasing age at diagnosis on average, the burden of melanoma to society has increased rapidly between 1989 and 2006, mostly due to increases in incidence rates.

The lifetime probability of an individual melanoma patient to die from their melanoma was low, implying that the majority of melanoma patients will live many years after their diagnosis (YLD for males: 18 per 100 000 men and for females: 31 per 100 000 women). The disability weight used in calculating the YLD was 0.05 [12], which is based on the prognosis of melanoma patients. Previous research has shown that more than a third of melanoma patients experience considerable levels of anxiety, mainly during diagnosis and treatment. [13] Moreover, patients' concerns may be very specific (e.g., in relation to UV exposure) and not be fully captured by generic health-related quality of life instruments. For example, a cross-sectional study among more than 500 melanoma survivors up to 10 years of diagnosis showed that most melanoma patients reported less frequent holidays to sunny destination compared to the times before their diagnosis and they also reported more anxiety for the deleterious effect of UV-light on their skin and more use of more protective measures, including practicing less hobbies outside and more protective clothing. Moreover, a proportion of melanoma survivors reported difficulty obtaining a life insurance or mortgage. These findings suggest that the YLD might not fully capture the actual years patients are living with their melanoma and its consequences; the disability weight should probably be raised to capture the true impact of melanoma on quality of life. Therefore, we calculated the Years Lived with Disease (YLWD), not taking the disability weight into account, as well and observed that a melanoma patient on average has to live 20 to 30 years with the impact of melanoma on their daily life.

To our knowledge we are the first to fully report on the burden of disease concepts in melanoma and to estimate the probability of being diagnosed with melanoma for the Dutch population. Dutch females had a probability of 1.6% of developing a melanoma during their lifetime, for males this was 1.2%. Calculating the probability of developing a cancer by estimating the cumulative risk does not take other comorbidities into account, nor the probability of dying from a disease other than melanoma. Therefore we calculated the risk of developing melanoma by the life table method using the DevCan program that calculates the probability of developing a melanoma and the probability of someone dying from it. These calculations were based on a hypothetical cohort and the estimated results of the DevCan analyses were confirmed by a standard life table. A life table makes it possible to answer simple questions of patients pertaining to their survival or the chance of developing a melanoma in the general population in certain age and sex groups. A persons' life time risk of developing a melanoma seemed relatively low, however the Dutch Cancer Society has shown before that this probability almost equals that of the chance for a women to develop ovarian cancer, non-Hodgkin lymphoma or lymphoma. [10]

Conclusion

In conclusion, the burden of melanoma is high and is increasing suggesting a need for adjusting health care policies to cope with this burden. Our research also shows that, even though a disease may be relatively rare and/or has a good prognosis, it can be associated with a great burden to individual patients' and society.

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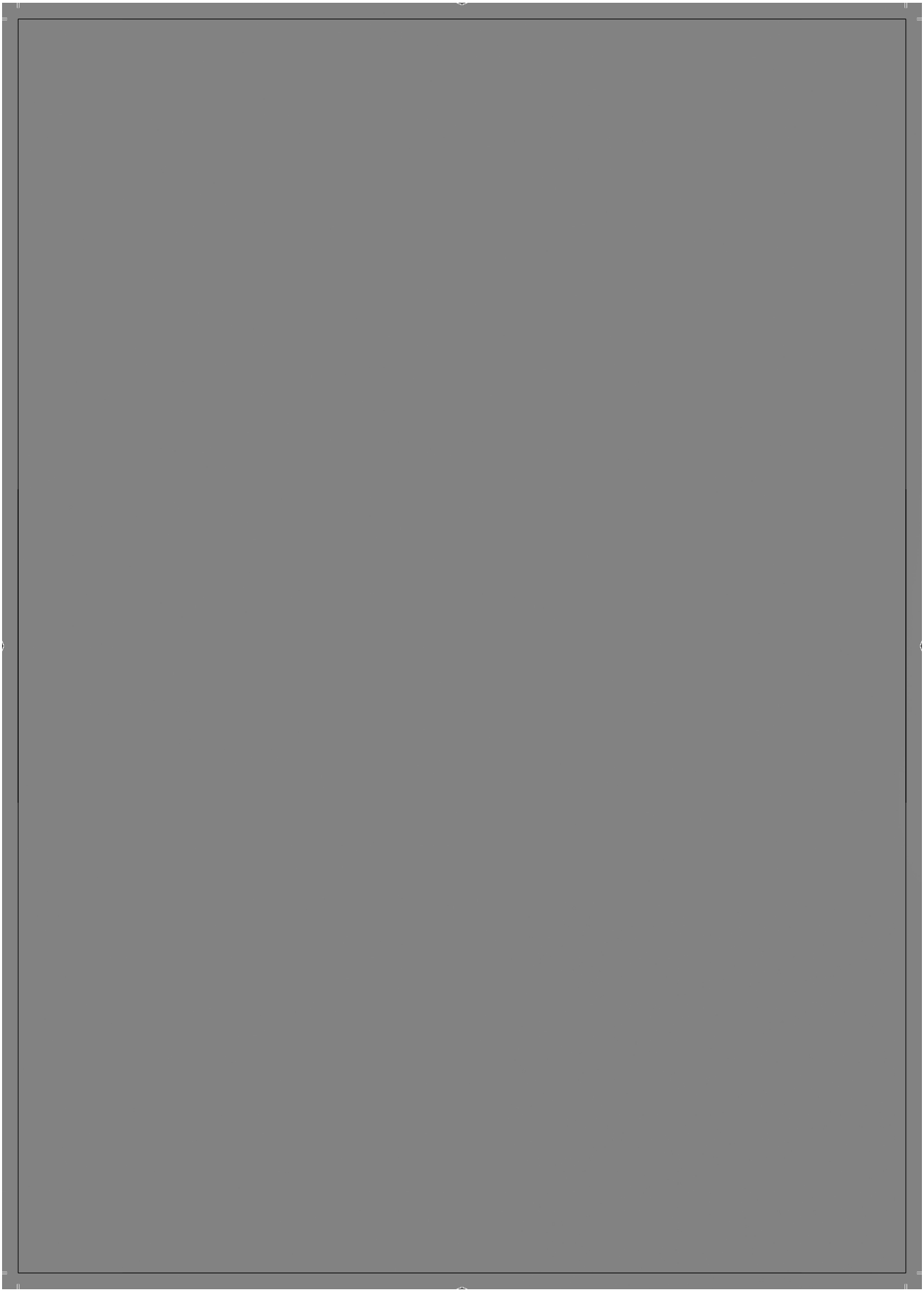


Chapter 4

Chemoprevention of melanoma

Chemopreventive drugs and their pharmacological mechanism of action, efficacy, safety and tolerability

4

Els R. Koomen, Tamar Nijsten, Henk-Jan Guchelaar

Submitted

Abstract

Background: In most countries, despite sun protection measures, the burden of melanoma is increasing. Therefore, melanoma chemoprevention may be a promising approach for high risk target populations. However, it is unclear which candidate drugs for chemoprevention of cutaneous melanoma have the potential to be useful and safe. Our aim was to systematically search the literature to identify candidate drugs for melanoma chemoprevention and to critically review their possible mechanism(s) of action, the existing evidence for their chemopreventive efficacy, as well as their safety and tolerability.

Methods: We conducted a systematic literature search in Medline, Embase, Web of Science and The Cochrane Library. Subsequently, we conducted a qualitative review on the potential chemopreventive drugs for which human data from clinical trials or observational research were available.

Results: Considerable evidence exists to suggest that melanoma development may be prevented or delayed by aspirin, NSAIDs and statins. Less evidence is available for other potential chemopreventive drugs, such as fibrates, retinoids, imiquimod, dehydroepiandrosterone, and acetaminophen. Long-term safety data in suitable chemopreventive dosages are not available for most these candidate drugs.

Conclusion: Although considerable preclinical evidence is available for aspirin, NSAIDs, and statins, in our opinion, there are still not sufficient (clinical) efficacy data and long-term safety data in chemopreventive dosages to perform a formal risk-benefit ratio and justify melanoma chemoprevention to move forward to current practice.

Abbreviations

ACTH	adrenocorticotropin
AJCC	American Joint Committee on Cancer
AK	actinic keratoses
APL	acute promyelogenous leukemia
APPROVe	Adenomatous Polyp Prevention on Vioxx
BCC	basal cell carcinoma
CDK	cyclin-dependent kinase
CDKI	cyclin-dependent kinase inhibitors
CI	confidence interval
CK	creatinine kinase
CNS	central nervous system
COX	cyclooxygenase
DAIS	Diabetes Atherosclerosis Intervention Study
DHEA	dehydroepiandrosterone
EMEA	European Medicines Agency
ERK	extra cellular signal-regulated kinase
FAMMM	Familial atypical multiple mole-melanoma
FDA	Food and Drug Administration
FFP	farnesyl pyrophosphate
FIELD	Fenofibrate Intervention and Event Lowering in Diabetes
FTI	farnesyl transferase inhibitors
GFR	glomerular filtration rate
GGP	geranylgeranyl pyrophosphate
GGTI	geranyl geranyl transferase inhibitors
GI	gastrointestinal
GPRD	General Practitioners' Research Database
GSH	glutathione
G-6-PD	glucose-6-Phosphate Dehydrogenase
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme-A
HR	hazard ratio
IFN	interferon
IL	interleukin
LFA1	lymphocyte function-associated antigen 1
LM	lentigo maligna
LMM	lentigo maligna melanoma

LSR	local skin reactions
MC1R	melanocortin-1 receptor
MEK	mitogen-activated protein kinase
NAC	N-acetylcysteine
NF-κB	nuclear factor-κB
NMSC	nonmelanoma skin cancer
NSAID	non-steroidal anti-inflammatory drug
OR	odds ratio
OTC	over the counter
PPAR	peroxisome proliferator-activated receptor
RA	retinoid acid
RAR	retinoic acid receptor
RCT	randomized clinical trial
ROS	reactive oxygen species
RR	relative risk
RXR	retinoid X receptor
SCC	squamous cell carcinoma
SCID	severe combined immunodeficient mice
SIR	standardized incidence rate
Th1	T helper cell type 1
TLR	toll-like receptor
TNF	tumor necrosis factor
TXA_2	thromboxane A_2
VIN	vaginal intraepithelial neoplasia
VITAL	Vitamins and Lifestyle
WHO	World Health Organization

Introduction

Melanoma incidence is rising steadily in most European countries as well as in Australia and in the US. [1] Although melanoma of the skin is usually diagnosed while confined to the local site / skin (AJCC stage I or II) and melanoma mortality rates seem to be stabilizing or even slightly decreasing in countries with high melanoma incidence rates [2], safe and effective treatment options for advanced stages of melanoma are still lacking making the prognosis for patients with advanced melanoma (AJCC stage III or IV) poor. [3] Thus, the burden of cutaneous melanoma is increasing. [4] Consequently, melanoma

prevention has high potential benefit and is increasingly the focus in melanoma research. Cancer prevention can be categorized into: 1) primary prevention of the initial cancer; 2) secondary prevention of invasive cancer in patients with premalignant conditions; and 3) tertiary prevention of second primary cancers. [5] As preventive measures for melanoma several strategies, mostly sun protection measures, have been suggested. However, even in countries where comprehensive sun protection programs have been in place for more than a decade and the use of sun screen is widely promoted, the incidence of melanoma is still rising. [6] Therefore, alternative approaches should also be considered and one of these alternatives could be chemoprevention.

Several definitions for the term 'chemoprevention' have been proposed. The term was first used in 1976 by Sporn and colleagues. They defined 'chemoprevention' as 'the use of natural or synthetic drugs to reverse, suppress, or prevent premalignant molecular or histological lesions from progressing to invasive cancer'. This also includes preventing in situ lesions to progress to invasive melanoma. [7]

Over the last decades, chemoprevention of cancer in general has gained interest and has resulted in a few first successes, such as tamoxifen in breast cancer, the first Food and Drug Administration (FDA)-approved chemopreventive drug, celecoxib for familial adenomatous polyposis and diclofenac and imiquimod for actinic keratosis. [8] Despite this 'proof of principle', adverse results appeared in chemoprevention trials hampering progress in cancer chemoprevention. For example, beta carotene has been associated with an increase rather than a reduction of the incidence of lung cancers [9], oral alfa-tocopherol supplementation resulted in an excess second primary head and neck cancers [10], and rofecoxib (Vioxx®, Merck) was withdrawn from the market after thrombotic cardiovascular events were observed in the APPROVe (Adenomatous Polyp Prevention on Vioxx) trial. [11] Indeed, these examples highlight the need for sound preliminary evidence of chemopreventive efficacy and also for a critical review of safety issues and the assessment of the overall risk-benefit ratio.

Specifically, chemoprevention of melanoma has gained interest in the recent years. Several epidemiological studies and clinical trials from different clinical settings may provide evidence for the chemopreventive efficacy of cutaneous melanoma. Associations between drug use and melanoma incidence from observational studies may help to test the hypotheses on chemopreventive activity. Clinical trials that may be of interest include: 1) cancer chemoprevention trials among healthy high risk individuals, 2) clinical trials in the non-oncology setting if incident cancers including melanomas were recorded as a secondary end point, 3) surrogate marker trials and 4) adjuvant melanoma trials. [8] Due to this broad range of sources of evidence, we believe the form of a true systematic review in this particular field would be restrictive and even inappropriate.

The aim of this qualitative review was to systematically search the literature to identify candidate drugs for chemoprevention of cutaneous melanoma, to critically review their possible mechanisms of action and to summarize the existing evidence for their chemopreventive efficacy, as well as safety and tolerability.

Methods

We define chemoprevention of melanoma as the use of natural or synthetic drugs to prevent, reverse, suppress or delay premalignant lesions from progressing into invasive cutaneous melanoma. This includes preventing in situ lesions from progressing to invasive melanoma.

Literature search

We searched Medline, Embase, Web of Science and The Cochrane Library (January 1st 1991 until April 12th 2008) using the search terms 'melanoma', 'chemoprevention', 'melanoma/prevention and control', 'chemoprophylaxis', 'chemicals and drugs category' and 'drug'. The complete search strings can be issued on request. Only manuscripts in English were included.

We selected scientific papers on drugs aimed for chemoprevention of cutaneous melanoma. Papers were excluded if they did not include cutaneous melanoma, did not meet the definition of chemoprevention, if there was no drug intervention (e.g., a non-pharmacological intervention) or if it was a non-scientific publication type.

Papers identified through cross referencing were as yet included if the studies concerned clinical trials or epidemiological research (meta-analyses, cohort studies or case control studies) generating evidence for chemopreventive activity in humans.

Drugs

We restricted our review to drugs for which human data were available from (randomized) clinical trials (RCT) or observational research, (i.e., meta-analyses, cohort studies or case-control studies).

Results

Search results

Our initial literature search resulted in 1158 references from Medline, Embase, Web of Science and The Cochrane Library (Fig. 1). In total, 1112 of these references were excluded; 619 because they focused on a non-pharmacological intervention (such as sun protection measures, vaccines or counseling), 152 because they did not include cutaneous melanoma, 300 because they did not meet the definition of chemoprevention, 32 because they were of one the following publication types: editorial, case report, letter or commentary, 4 because they were not published in English and 5 because no studies with human data were available on this (group of) drug(s). Additionally, 131 papers were identified through cross referencing, were as yet included.

General remarks

Potential Chemopreventive Drug Classes

The potential chemopreventive drugs that resulted from our systematic literature search were: non-steroidal anti-inflammatory drugs (NSAIDs, including selective cyclooxygenase-2-inhibitors and aspirin), statins, fibrates, retinoids, imiquimod, dehydroepiandrosterone (DHEA), acetaminophen, apomine, capsaicin, urokinase receptor antagonists, N-acetylcysteine, farnesyl transferase inhibitors (FTIs), and geranyl geranyl transferase inhibitors (GGTIs).

For apomine, capsaicin, urokinase receptor antagonists, N-acetylcysteine, FTIs, GGTIs, we did not find any human efficacy data on melanoma chemoprevention from observational research or clinical trials. Consequently, this review focused on NSAIDs, statins, fibrates, retinoids, imiquimod, DHEA, and acetaminophen.

Prerequisites

Prerequisites and requirements for research in melanoma chemoprevention and for a valid melanoma strategy have been defined earlier by Demierre, Nathanson, Merlino and Sondak (Table 1). [8;12-14]

From the clinical viewpoint, it requires:

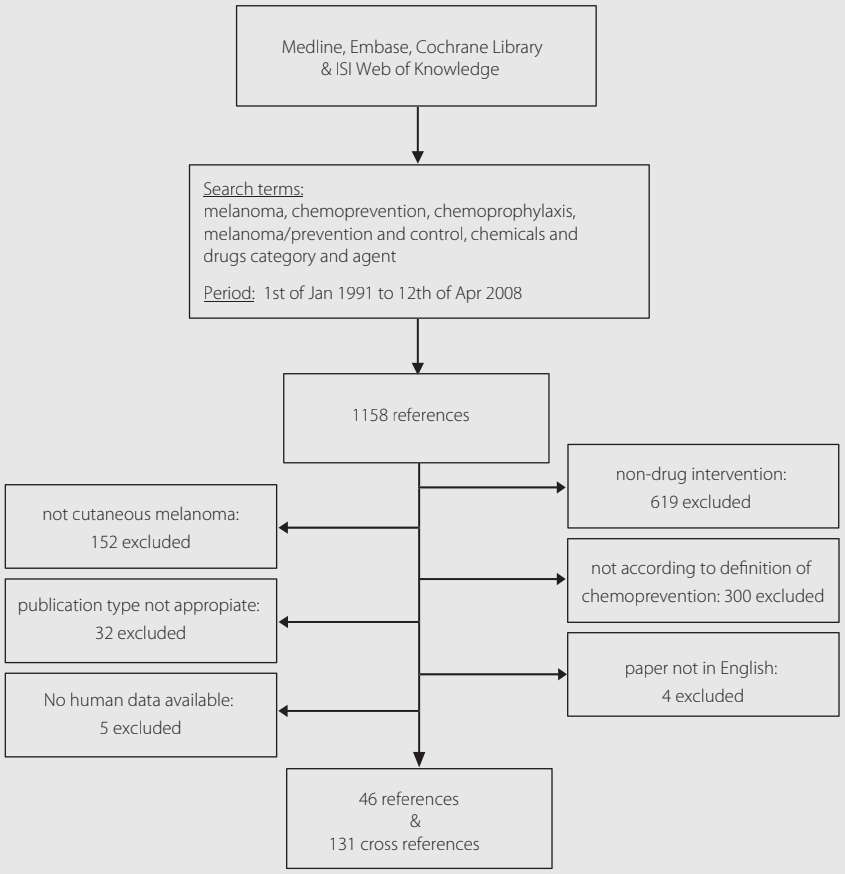
(1) chemopreventive drug efficacy;

(2) acceptable safety & tolerability;

(3) effectiveness in clinical practice, and

(4) a large potential benefit for the chemoprevention target population.

Figure 1



Total exluded:	1112	(100%)
- non-pharmacological intervention	619	(55.7%)
- not cutaneous melanoma	152	(13.7%)
- definition of chemoprevention	300	(27.0%)
- publication type	32	(2.9%)
- paper not in English	4	(0.4%)
- no human data available	5	(0.4%)

Table 1 Prerequisites for progress in cancer chemoprevention research

Prerequisite	Requirements
Elements of a strong scientific rationale	(i) Determination of the underlying molecular mechanisms of carcinogenesis (ii) Discovery of genetic markers that identify the early events in the carcinogenic process (iii) Availability of drugs that can target the molecular mechanism of carcinogenesis
Long-term safety of candidate drugs	(i) Availability of long-term human safety data (ii) Availability of animal tumor models that permit preclinical trials of evaluation of drug toxicity
Critical elements of a rigorous chemoprevention clinical trial design	(i) Availability of animal tumor models that permit preclinical trials of evaluation of drug efficacy (ii) Compilation of data from epidemiologic, basic science, and cancer research literature that can yield candidate prevention drugs for in-vitro or in-vivo testing (iii) Availability of molecular or histologic markers of the carcinogenic process to be used as endpoints and to obviate the need for prolonged and costly trials (iv) Access to defined groups at very high risk for the disease

From: Demierre MF. What about chemoprevention for melanoma? Curr Opin Oncol 2006 Mar;18(2):180-4.

Ad 1. Obviously, a strong scientific rationale and proven efficacy of the chemopreventive drug is required. As Demierre and Nathason described earlier [8], efficacy should be demonstrated in *in vitro* research, validated animal models, such as transgenic murine models. Additionally, efficacy must be observed in humans at (high) risk of a (second) invasive melanoma. Human efficacy data should include well designed phase I and II chemoprevention studies, and finally full-scale phase III trials. [15-17] These phase III trials should be designed to include endpoints to evaluate both expected and unexpected adverse events to allow full evaluation of the risk-benefit ratio.

Ad 2. In melanoma chemoprevention, healthy individuals at high risk of developing melanoma are the target population. Thus, there is no direct therapeutic effect. Moreover, chemopreventive drugs are frequently given for at least 5 years during which adherence to the drug regimen must be maintained. Little-to-no toxicity is, therefore, an absolute prerequisite to ensure both long-term safety and compliance.

A well-established safety profile may exist for drugs already marketed for alternative indications. However, higher drug dosages and longer treatment durations may be required for (melanoma) chemoprevention. Moreover, the distribution of risk factors for potential adverse events may differ between the target populations of these indications. Thus, a drug that appears to be safe for one indication may not be considered sufficiently safe for the use in cancer chemoprevention. Ideally, a chemopreventive drug would have additional major health benefits on high-prevalent diseases or health outcomes.

Ad 3. Efficacious drugs may not be effective in clinical practice. A possible explanation is lack of adherence to the drug regimen. Important prerequisites for adherence are likely to be little-to-no toxicity of the drug and a sufficiently motivated target population

Ad 4. It should be clear-cut for which patients the chemopreventive drug would be indicated. Because the absolute risk of getting a melanoma is small, chemoprevention should be targeted at patients at high risk of developing an invasive melanoma. To define the high risk populations that would benefit from chemoprevention, validated prediction models are warranted.

Target population

Well-established risk factors for melanoma are history of sun burns, older age, clinical atypical nevi, prior melanoma, family history of melanoma (FAMMM) or mutational status (CDKN2A/p16^{INK4A} mutations, CDK4 mutations, MC1R variants), and phenotypic traits, such as fair skin type, freckles, light eye color and photosensitivity. Among these, the validated and strongest predictors of melanoma incidence are likely to be suitable for the selection of a chemoprevention target population.

Possible high risk populations to target could be patients with prior melanoma, individuals with a family history of melanoma and clinical atypical nevi, individuals with multiple clinical atypical nevi and/or patients with atypical mole syndrome. [18-21] Future advances in research on validated prediction models and biomarkers, will hopefully increase possibilities for more specific definitions of high risk groups on whom melanoma chemoprevention should target.

Non-steroidal Anti-inflammatory Drugs

NSAIDs are traditionally prescribed because of their analgesic, antipyretic and anti-inflammatory effects. NSAIDs inhibit the cyclooxygenase (COX) enzyme reversibly leading to reduced synthesis of prostaglandins and thromboxane.

Based upon their pharmacological effects, NSAIDs can be subdivided in three groups.

First, traditional NSAIDs, e.g. diclofenac, naproxen, sulindac, indomethacin, and piroxicam, reversibly inhibit both the constitutively expressed COX-1 and the inducible COX-2 isoform of the enzyme (i.e, nonselective COX-inhibitors). Secondly, the selective COX-2-inhibitors, e.g. celecoxib, etoricoxib, and rofecoxib, in regular doses, inhibit only the COX-2-isoform. Aspirin forms the third group because it irreversibly inactivates COX-1 by acetylating a serine residue in its active site and, therefore, reduces thromboxane A_2 (TXA_2) in platelets. Due to the fact that platelets cannot synthesize new enzyme, TXA_2 synthesis does not recover until new platelets arise after 7-10 days.

Mechanism of action

Overexpression of COX, especially COX-2, has been demonstrated in human cancer cells of several tumor types. Based upon these observations, the COX-pathway is hypothesized to be involved in carcinogenesis. Indeed, the *ras* oncogene stimulates and *p53*, a tumor suppressor, down-regulates COX-2 expression. Moreover, COX-2 expression also seems to enhance metastatic potential of colon cancer cells and may be involved in resistance to chemotherapeutic drugs. [22] Thus, the primary potential mechanism of action of NSAIDs in cancer chemoprevention is considered to be COX inhibition (Table 2). [23]

Increased COX-2 expression has been noted in the majority, but not all, melanoma cell lines. [24-26] Denkert *et al.* showed that five melanoma cell lines (A375, MeWo, SK-Mel-13, SK-Mel-28, and IGR-37) and 26 out of 28 (93%) patient derived primary melanomas showed COX-2 expression, whereas benign nevi (n=4) and epithelial cells were negative. After introduction of a COX-2 blocking agent, NS-398, cell line growth and invasive potential were inhibited. [24] Similarly, in a series of 101 ex vivo melanoma, 96 (95%) showed COX-2 expression. More importantly, in this study, the level of COX-2 expression was also negatively associated with disease-specific survival ($p = 0.046$). [25] Increasing evidence suggests that NSAIDs inhibit tumor growth and invasion [24;27;28] and can induce apoptosis [28;29]. Roh and colleagues demonstrated an inhibitory effect of both celecoxib and indomethacin on melanoma cell growth in a murine B16F10 melanoma model. [30] Also, in a study of human A-375 melanoma cells, incubations for 72-hour of 50 and 100 μM of celecoxib showed reduced proliferation. Additionally, in a Toxilight TU-cytotoxicity assay, 100 μM celecoxib was toxic to the cancer cells. In this experiment, indomethacin (240 and 480 μM) also inhibited cell proliferation, but was only slightly toxic. Neither aspirin nor piroxicam exhibited cytostatic or cytotoxic effects. Thus, of the tested NSAIDs (aspirin, indomethacin, piroxicam and celecoxib), only celecoxib and indomethacin reduced proliferation. Because these NSAIDs all inhibit COX-2 in these concentrations, the authors suggested

Table 2 Chemopreventive drugs, their potential mechanism of action, side effects and safety profile

Drug	Chemopreventive Mechanism(s)	In vitro effects	Side Effects	Health benefits	Improvement of risk-benefit ratio
NSAIDs[1]	*COX dependent*: • inhibited COX-2 expression • inhibition of PG synthesis *COX independent*: • LOX-metabolism • apoptotic genes • activation of caspases • p38 MAP kinase activation • mitochondrial cytochrome c • ceramide pathway activation	▪ inhibition of tumor growth ▪ apoptosis ▪ inhibition of invasiveness	▪ duodenal/gastric ulcers ▪ GI bleeding ▪ decreased renal function ▪ cardiovascular events ▪ cerebrovascular events	▪ no general extra health benefits	▪ *H. pylori* eradication and/or adding PPI to prevent ulcers ▪ exclude patients with decreased renal function / users of ACE inhibitors ▪ exclude patients with cardiovascular risk factors
Aspirin	• see NSAIDs *Additional COX independent*: • thrombocyte-aggregation • NF-κB • DNA-repair • oxidative stress • mitochondrial Ca^{2+}-uptake	▪ inhibition of tumor growth ▪ apoptosis ▪ inhibition of invasiveness	*Low-dose*: ▪ GI bleeding ▪ cerebrovascular bleeding *High dose*: ▪ duodenal/gastric ulcers ▪ GI bleeding ▪ decreased renal function	▪ prevents thrombotic cardiovasular and cerebrovascular events	▪ *H. pylori* eradication and/or adding PPI to prevent ulcers ▪ High dose aspirin: exclusion of patients with decreased renal function / users of ACE inhibitors
Statins	*Inhibition HMG-CoA reductase*: Prevent prenylation of: • RhoA, RhoC and Ras, and other prenylation-dependent proteins *Cholesterol independent*: • binding to LFA1 • inhibition of the proteasome • increased fibrinolytic activity	▪ inhibition tumor growth by cell cycle arrest and apoptosis ▪ reduced invasiveness by inhibiting migrating factors & reducing adhesion molecules ▪ effects on angiogenesis ▪ attenuation of resistance mechanisms	▪ myopathy ▪ elevated CK levels ▪ rhabdomyolysis ▪ anorexia ▪ nausea ▪ diarrhea ▪ fatigue ▪ ulcerative lesions	▪ prevents cardiovascu-lar events ▪ potential positive effects in osteoporosis and Alzheimer's disease	▪ High dosages: contraindicated in presence of relative renal dysfunction (CLcr < 60-70 ml/min) ▪ adding ubiquinone to prevent statin-induced myopathy ▪ prevent concomitant drug use with gemfibrozil, CYP3A4 or CYP2C9 inhibitors[2]

Fibrates	▪ PPAR-α or PPAR-γ agonism ▪ direct toxic effect of low cholesterol on malignant cells	▪ inhibition of tumor growth ▪ apoptosis ▪ antimetastatic effects	▪ abdominal pain/dyspepsia ▪ increased creatinine/urea ▪ myopathy ▪ elevated CK levels ▪ rhabdomyolysis ▪ increased homocysteine ▪ cholelithiasis ▪ (venous thrombosis) ▪ (pulmonary emboli)	▪ prevents cardiovascu-lar events ▪ potentially reduces proteinuria in diabetes patients	▪ adjusted fibrate dosing or contraindication if renal function is decreased (CLcr < 50 ml/min); does not apply for gemfibrozil
Retinoids	▪ RXR or RAR-α, β, or γ binding leading to altered gene transcription *RAR & RXR independent:* ▪ inhibition of mitogen-induced c-fos expression ▪ Rac-dependent ROS increase ▪ increased expression of p16, p21, p27, p53, and bax ▪ MAPK, Bcl-2 down-regulated	▪ inhibition of tumor growth ▪ apoptosis ▪ proangiogeneic effects ▪ antimetastatic effects	*Topical treatment*: ▪ Skin irritation *Oral treatment*: ▪ teratogenicity ▪ bone toxicity ▪ hepatotoxicity ▪ serum lipid abnormalities ▪ cheilitis, xerosis ▪ ocular effects ▪ hair loss	▪ no general extra health benefits	▪ combining topical retinoid with topical hydrocortisone to control skin irritation ▪ contraindicated during pregnancy or lactation ▪ avoid use among women of child bearing age ▪ use contraceptive measures required ▪ pregnancy test prior to start of therapy
Imiquimod	▪ TRL7 stimulation induces a Th1 immune response which results in transformation of naïve T cells ito antigen-specific T cells directed against antigens expressed on potentially immunogenic skin tumors	▪ inhibition of tumor growth ▪ apoptosis	▪ skin irritation ▪ sun sensitivity ▪ allergy ▪ headache ▪ muscle weakness ▪ fever & flu-like symptoms ▪ fungal infection	▪ no general extra health benefits	
Acetaminophen	▪ GSH depletion leading to ROS formation and mitochondrial toxicity ▪ may act as tyrosinase substrate	▪ inhibition of tumor growth ▪ cytotoxic effects in high doses (?)	▪ urticarial rash ▪ allergic reactions ▪ renal failure (chronic use) *Very high doses*: ▪ nausea, vomiting ▪ hyperglycaemia ▪ liver failure	▪ no general extra health benefits	▪ high doses: NAC infusion ▪ exclude patients with G6PD deficiency

NSAID = non steroidal antiinflammatory drug, COX = cyclooxygenase, PG = prostaglandin, LOX = lipoxygenase, MAP = mitogen-activated protein, PPAR = peroxisome proliferator-activated receptor, RAR = retinoic acid receptor, RXR = retinoid X receptor, ROS = reactive oxygen species, TRL = Toll-like receptor, GI = gastrointestinal, *H. pylori* = Helicobacter pylori, PPI = proton pump inhibitor, ACE = Angiotensin Converting Enzyme, CK = creatinine kinase, CLcr = creatinine clearance, NAC = N-acetylcysteine.
[1] Both traditional NSAIDs and COX-2-inhibitors. Note: cardiovascular events are more prevalent among users of selective COX-2-inhibitors and duodenal/gastric ulcers & GI bleedings are less prevalent. [2] For atorstatin, lovastatin, cerivastatin or simvastatin, concomitant use of CYP3A4 inhibitors (e.g., grape fruit juice, itraconazole, ketoconazole, nelfinavir, indinavir, ritonavir, erythromycin, verapamil) should be avoided. For fluvastatin, concomitant use of CYP2C9 inhibitors (e.g., fluconazole, amiodarone) should be avoided. Increased risk of myopathy and rhabdomyolysis if a statin is combined with gemfibrozil.

that the growth inhibitory effect of celecoxib cannot be explained solely by its COX-inhibitory activity. [27]

Additional COX-independent pathways have also been suggested in other cancer types. [31;32] Numerous possible targets, such as lipoxygenase metabolism (ALOX15) [33], the proapoptopic gene *PAWR* [34], the anti-apoptopic gene *BCL2L1* [35], activation of caspases {36], the activation of p38 MAP kinase [37], release of mitochondrial cytochrome c [38], and activation of the ceramide pathway [39], have been suggested to be involved. These COX-independent pathways, however, need further study. For example, some investigators have suggested that only higher aspirin doses lead to these COX-independent molecular mechanisms. [40] Moreover, aspirin may have additional anticancer pathways as compared to other NSAIDs, such as inhibition of thrombocyte-aggregation [41], NF-κB, DNA-repair systems, apoptosis, oxidative stress or mitochondrial calcium uptake [31].

Evidence for efficacy in humans

Although some studies were promising, conflicting results exist on NSAIDs in melanoma prevention (Table 3). Initially, Harris *et al.* reported a small case control study (110 cases, 609 controls, all females) in which regular NSAID use showed a significantly decreased relative risk (RR) of melanoma (RR = 0.45 with a 95% confidence interval (CI) of 0.22 to 0.95). With increasing NSAID use, melanoma risk further decreased (*p*-linear trend <0.05). Estimates for daily use of aspirin were similar (RR = 0.55). [42]

Subsequently, in a small retrospective cohort study of 83 melanoma patients, users of NSAIDs or COX-2-inhibitors, as compared to nonusers, had a lower incidence of new melanoma, recurrence, and metastasis (combined end point; odds ratio (OR) of 0.08, 95% CI = 0.01-0.77). [43] However, we believe guarantee-time bias may have importantly influenced these results. In explanation, NSAID exposure in this study was defined as any prescription after first diagnosis of melanoma and prior to development of a new melanoma, a recurrence or metastatic lesion. Consequently, patients with longer survival are more likely to be categorized as a NSAID user due to the simple fact that their follow-up period was longer. More complex study designs and statistical analyses could have prevented such bias. [44]

In a secondary analysis of the Women's Health Study, Cook and colleagues studied low-dose aspirin (100 mg every other day) versus placebo. Among the 39,885 women included in this RCT, low-dose aspirin was not associated with melanoma risk (RR = 0.97, 95% CI = 0.70-1.36). [45] Similar results were obtained in a secondary analysis of the Cancer Prevention Study II Nutrition Cohort. Although long-term adult-strength

aspirin (≥325 mg for ≥5 years) was associated with lower overall cancer incidence in men and a non-statistically significant lower overall cancer incidence was observed in women, melanoma incidence was not reduced (current daily use, ≥5 years: RR = 1.15, 95% CI = 0.83-1.59, <5 years: RR = 0.99, 95% CI = 0.79-1.25). [46]

Recently, in the Vitamins and Lifestyle (VITAL) cohort study, Asgari *et al.* examined the association between NSAID use and melanoma risk. Among 63,809 men and women, during a 10 year follow-up period, 349 patients with incident melanomas were identified including 157 in situ melanomas. Use of any NSAID for at least 4 days per week as compared to nonuse, did not seem to reduce the melanoma hazard rate (HR; HR = 1.12, 95% CI = 0.84-1.48). Similar results were obtained for any NSAID excluding low-dose aspirin (HR = 1.03, 95% CI = 0.74-1.43), for regular- or extra-strength aspirin (HR = 1.10, 95% CI = 0.76-1.58), and for nonaspirin NSAIDs (HR = 1.22, 95% CI = 0.75-1.99). Additionally, NSAID use was not associated with tumor invasion (*p*-interaction = 0.38), tumor thickness (*p*-linear trend = 0.98), or risk of metastasis (HR = 1.09, 95% CI = 0.32-3.62). [47]

In a large population-based case control study of our group including 1,318 patients with invasive melanoma and 6,786 controls, incident melanoma was not associated with aspirin use (OR = 0.92, 95% CI = 0.76-1.12) or non-aspirin NSAID use (OR = 1.10, 95% CI = 0.97-1.24). However, continuous use of low-dose aspirin was associated with a significant reduction of melanoma risk in women (OR = 0.54, 95% CI = 0.30-0.99) but not in men (OR = 1.01, 95% CI = 0.69-1.47). A significant linear trend ($p = 0.04$) from non use, non-continuous use, to continuous use was observed in women. [48]

Recently, the Harvard Cancer Center performed a case control study among 400 melanoma patients and 600 matched community based controls. After adjusting for confounders, use of any NSAID, at least once weekly for more than 5 years as compared to use for less than 2 years, was associated with an adjusted OR of 0.55 (95% CI = 0.42-0.77). For aspirin and non-aspirin NSAIDs the odds ratios were comparable (OR = 0.51, 95% CI = 0.35-0.75 and OR = 0.64, 95% CI = 0.46-0.89, respectively). If NSAID use was defined as any use versus no use, the results were somewhat less pronounced (*personal communication*).

Specific studies on selective COX-2 inhibitors are lacking. Duke and colleagues have planned a Cochrane review 'COX-inhibitors in the prevention of melanoma'. [49] If enough eligible trials will be pursued, this review will likely provide more insight.

In summary, due to heterogeneity in study design (ascertainment and definition of exposure, type of NSAID, dose, duration, patterns of use, drug adherence, study population etc), conflicting results and the limited number of studies, the efficacy of NSAIDs and aspirin for melanoma prevention remains unclear. The results of *in vitro*

Table 3 Associations between use of potential chemopreventive drugs and incident melanomas

Drug	Design	Numbers	Dose	Duration of use	Follow up	Estimate[1]	95% CI	Primary endpoint	Ref	Remarks
NSAIDs, all	CO	N= 63,809	≥ 4 d/wk	NR	5 y [2], 1-10 y [3]	HR=1.12	0.84 - 1.48	no	1391	MM: N = 348
	CC	N= 400 MM N= 600 C	≥ 1 PPW	>5 y vs. <2 y	n.a.[4]	OR=0.73	0.55 – 0.97	yes	*	
	CC	N= 101 MM N= 609 C	≥ 1 PPD	≥ 2 y	-	OR=0.45	0.22 - 0.92	yes	260	only females
	CC	N= 101 MM N= 609 C	< 1 PPD	≥ 2 y	-	OR=0.77	0.35 - 1.70	yes	260	only females
Non-aspirin	CO	N= 63,809	≥ 4 d/wk	NR	5 y [2], 1-10 y [3]	HR=1.12	0.85 - 1.49	no	1391	MM: N = 348
	CC	N= 1,318 MM N= 6,786 C	No dose limit	≥ 1/2 y	3 y (100%)	OR=1.10	0.97 - 1.24	yes	1487	
	CC	N= 400 MM N= 600 C	≥ 1 PPW	>5 y vs. <2 y	n.a.[4]	OR=0.64	0.46 – 0.89	yes	*	
Aspirin	RCT	N= 19,942 P N= 19,934 A	100 mg qod	NR [5]	10.1 y [2]	RR=0.97	0.70 - 1.36	no	1434	only females MM: N = 138
	CO	N= 146,113	≥ 325 mg qd	max. 11 y	≥ 5 y	RR=1.15	0.83 - 1.59	no	1435	MM: N = 871
	CO	N= 146,113	≥ 325 mg qd	max. 11 y	< 5 y	RR=0.99	0.79 - 1.25	no	1435	MM: N = 871
	CO	N= 63,809	≥ 325 mg ≥ 4 d/wk	NR	5 y [2], 1-10 y [3]	HR=1.10	0.76 - 1.58	no	1391	MM: N = 348
	CC	N= 1,318 MM N= 6,786 C	≤ 100 mg qd	≥ 1/2 y	3 y (100%)	Males: OR=1.01 Females: OR=0.54	Males: 0.69 - 1.47 Females: 0.30 - 0.99	yes	1487	stratified for sex (prespecified)
	CC	N= 1,318 MM N= 6,786 C	> 100 mg qd	≥ 1/2 y	3 y (100%)	OR=1.35	0.96 - 1.92	yes	1487	

	CC	N= 400 MM N= 600 C	≥ 1 PPW	>5 y vs. <2 y	n.a.[4]	OR=0.51	0.35 – 0.75	yes	*	
	CC	N= 101 MM N= 609 C	≥ 1 PPD	≥2 y	-	OR=0.55	NR	yes	260	only females
Retinoids	CO	N= 162,000	≥1.8 vs. <0.4 mg/d	max. 8-14 y	max. 8-14 y	RR=0.39	0.22 – 0.71	no	726	MM: N = 414 only reviewers biopsies blinded
	CC	N= 542 MM N= 538 C	highest vs. lowest quartile	NR	NR	OR=0.57	0.39 – 0.83	yes	138	
Acetami-nophen	CO	N= 39,946	No dose limit	NR	4.7 y [2], 1-9 y [3]	SIR=0.9	0.6-1.2	no	1469	MM: N = 39 NSAID and aspirin use included
	CO	N= 13,482	No dose limit	NR	4.7 y [2], 1-9 y [3]	SIR=0.6	0.2-1.3	no	1469	MM: N = 7 NSAID and aspirin users excluded
	CC	N= 101 MM N= 609 C	≥ 1 PPD	≥ 2 y	-	OR=0.95	0.45-1.98	no	260	only females matched on age and place of residence
Statins	RCT	N= 2,223 P N= 2,221 S	10-40 mg qd	ITT	5.4 y [2]	RR=2.34	0.60 – 9.06	no	16	4S study MM: N=7 S / 3 P
				(5.4 y [2] ITT)	10.4 y	RR=1.28	NR	no	1470	Follow up 4S study MM: N=9 S / 7 P
	RCT	N= 10,267 P N= 10,269 S	40 mg qd	ITT	4.6 y [2]	RR=1.66	0.78 - 3.54	no	1467	HPS study MM: N=17 S / 10 P
	RCT	N= 3,301 P N= 3,304 L	20-40 mg qd	0.2-7.2 y [3]	5.2 y [2]	OR=0.52	0.27 – 0.99	no	1399	AFCAPS study MM: N=14 L / 27 P
	RCT	N= 2,078 P N= 2,081 Pr	40 mg Pr qd	NR [6]	5 y [7]	OR=1.33	0.30 – 5.96	no	35	CARE study MM: N=4 Pr / 3 P
	RCT	N= 4,502 P N= 4,512 Pr	40 mg Pr qd	ITT	6.1 y [2]	OR=1.07	0.64 – 1.79	no	16	LIPID study MM: N=30 Pr / 28 P

Table 3 Continued

Drug	Design	Numbers	Dose	Duration of use	Follow up	Estimate[1]	95% CI	Primary endpoint	Ref	Remarks
Statins	RCT	N= 3,293 P N= 3,302 Pr	40 mg qd	ITT	4.9 y [2]	OR=0.66	0.19 – 2.36	no	16	WOSCOP study males only MM: N=4 Pr / 6 P
	RCT	N= 1,049 P N= 1,045 F	40 mg qd [8]	ITT	5.1 y [2]	RR=0.40	NR	no	1468	ALERT trial MM: N=2 F / 5 P
	CC	N= 79 MM N= ~ 395 C	No dose limit	current use	6.4 y [7]	RR=2.5	0.8 - 7.3	yes	1400	GPRD database
	CC	N= 1,318 MM N= 6,786 C	No dose limit	≥ 1/2 y	3 y (100%)	OR=0.98	0.78 - 1.2	yes	1003	
Fibrates	RCT	N= 1,542 P N= 1,548 B	400 mg qd	ITT	6.2 y [2]	OR=0.33	0.07 – 1.64	no	16	BIP study MM: N=2 B / 6 P
	RCT	N= 785 P N= 783 B	400 mg qd [9]	ITT	4.6 y [7]	OR=1.00	0.06 – 16.1	no	16	LEADER study males only MM: N=1 G / 1 P
	RCT	N= 2,030 P N= 2,051 G	600 mg bid	ITT	5 y [2]	OR=2.97	0.12 – 73.0	no	1461	HHS study males only MM: N=1 G / 0 P
	RCT	N= 1,267 P N= 1,264 G	1200 mg qd	ITT	5.1 y [7]	OR=0.11	0.01 – 0.87	no	1403	VA-HIT study males only MM: N=1 G / 9 P
	RCT	N= 2,789 P N= 1,103 C	1.8 g qd	NR	6.2 y [2]	OR=1.69	0.28 – 10.1	no	16	CDP study MM: N=2 C / 3 P

RCT=Randomized Controlled Trial, CO=Cohort study, CC= Case-Control study, N=number, MM=melanoma cases, C=controls, P=placebo, A=aspirin, S=simvastatin, L=lovastatin, Pr=pravastatin, B=bezafibrate, C=ciprofibrate, G=gemfibrozil, d/wk=days per week, mg=milligram(s), g= gram(s), PPD=pills per day, PPW=pills per week, mg/d = milligram per day, qd= once a day, qod=every other day, bid=twice a day, y=year(s), ITT=intention to treat, RR=relative risk, OR=Odds Ratio, HR=Hazard Ratio, SIR=Standarized Incidence Ratio, CI=confidence interval, NR=not reported, Ref=reference (see referencelist).

* Personal communication on a case control study among 400 melanoma patients and 600 matched community based controls (Harvard Cancer Center, T. Nijsten).

and animal studies, however, are promising. A pivotal unresolved problem is the definition of the temporal and dose-response cause effect relationships between NSAID use and incident invasive melanoma. Thus, additional experimental and observational research is warranted, particularly on required dosages and duration.

Safety, Tolerability & Compliance

Side effects of NSAIDs are gastrointestinal (GI) complaints, such as nausea, vomiting, dyspepsia (10-20%), diarrhea, duodenal or gastric ulcers (10-30%), sometimes even leading to GI bleedings or perforation (± 2%). [50] In addition, skin reactions, cardiovascular and cerebrovascular events, and decreases in renal function also occur. Rare, but serious, side effects are bone marrow disturbances and hepatotoxicity. The prevalence of GI related side effects differs substantially between several traditional NSAIDs, being less pronounced for aspirin and diclofenac compared to piroxicam.

COX-2-inhibitors have been developed to selectively inhibit COX-2 and thus to reduce side effects related to COX-1-inhibition, most importantly duodenal and gastric ulcers. Indeed, duodenal or gastric ulcers are less prevalent (± 2%) for this class of NSAIDs. [50] However, thrombotic cardiovascular events observed in the APPROVe trial, a chemopreventive trial in which patients with a history of colorectal adenomas were randomized to receive rofecoxib or placebo [11], have raised safety concerns regarding the risk-benefit ratio of COX-2-inhibitors in cancer chemoprevention. [51;52] Subsequent epidemiological studies have suggested that these events are also associated with traditional NSAIDs, such as ibuprofen or diclofenac. [53;54] In these studies, naproxen, as an exception, is associated with a reduced cardiovascular event rate. [53;54]

To prevent GI ulcers and bleeds, additional interventions such as *Helicobacter pylori* eradication and concomitant use of a proton pump inhibitor to the chemopreventive strategy could be considered, but this introduces new adverse effects and additional costs. Currently, in the AspECT trial a combination of aspirin plus proton pump inhibitor is studied for the chemopreventive activity on cancer among patients with Barret's esophagus. [55]

Aspirin may also cause bleeding through inhibition of thrombocyte-aggregation. Due to this feature, however, aspirin does not cause an

[1] multivariable adjusted estimates (RR, OR, HR) as reported in original publication.
[2] mean value presented.
[3] range value presented.
[4] not applicable.
[5] compliance, defined as taking ≥ 2/3 of the study drugs, was 76% at 5 years and 67% at 10 years.
[6] in the last year of follow-up, 86% of the placebo group and 94 percent of the treatment group were taking their study medication.
[7] median value presented.
[8] randomized to 40 mg fluvastatin or placebo. In both arms open label prescriptions of an additional 40 mg fluvastatin were allowed if cholesterol levels were too high.
[9] 400 mg qd or 400mg qod if creatinine 135-149 mmol/L.

excess of cardiovascular events and actually has the advantage of protection against cardiovascular disease. Moreover, aspirin may have additional chemopreventive effects as compared to other COX-inhibitors. [31;41] Nevertheless, due to the lack of definitive evidence on (differences in) efficacy, required dosages and duration, it is too early to claim aspirin as the preferential NSAID for cancer chemoprevention.

Conclusion Non-steroidal Anti-inflammatory Drugs

In vitro studies demonstrate COX-2-expression in melanoma and suggest effects of NSAIDs on growth inhibition, invasiveness and apoptosis. COX independent pathways, however, may also be involved in these anti-tumor effects. These pathways should be further investigated in order to disentangle dose-response relationships and identify the most promising NSAIDs. Although promising efficacy data were shown in other cancers, NSAIDs have yet to demonstrate sufficiently convincing evidence for efficacious melanoma chemoprevention. Convincing evidence is lacking and comparing the conflicting results of the limited number of published studies is challenging due to heterogeneity in study design and uncertainties in temporal and dose-response relationships. Moreover, concerns over the long-term safety of COX-2 inhibitors and NSAIDs have tempered the enthusiasm for their use in chemoprevention. Therefore, if sufficient data on efficacious drug dosages and temporal cause effect relationships become available, formal risk-benefit analyses should be performed on different scenarios of chemopreventive strategies.

Statins

Statins, or 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase inhibitors, are widely prescribed to reduce cholesterol levels aiming to prevent cardiovascular events. This drug class consists of atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, simvastatin, pitavastin, pravastatin, and rosuvastatin. Cerivastatin, however, has been withdrawn from the market in 2001 due to reports of rhabdomyolysis, especially with concomitant use of gemfibrozil.

Statins differ in several aspects. For example, lovastatin, simvastatin, and pravastatin were originally derived from fungi, whereas atorvastatin and fluvastatin are synthetically derived. Additionally, some statins are prodrugs, e.g. simvastatin and lovastatin, and have a closed lactone ring that is converted by carboxyesterases to the open-ring acid form that inhibits HMG-CoA reductase. [56]

Historically, an inverse association between cholesterol and the incidence of (smoking-related) cancers has been observed [57], suggesting a link between low cholesterol and cancer. In addition, lovastatin and gemfibrozil were shown to promote development

of liver cancer in rodents. [58] However, subsequent research demonstrated paradoxical results suggesting decreased cancer incidences with use of lipid-lowering drugs.

Mechanism of action

The putative mechanism of action for both the cholesterol lowering and anticancer effects of statins is considered to be inhibition of HMG-CoA reductase, an enzyme upstream in the mevalonate biosynthetic pathway. Inhibition of HMG-CoA reductase leads to reduced synthesis of mevalonate and its downstream products. Farnesylpyrophosphate (FPP), a C_{15}-moiety, is one of these downstream products and is the precursor of both geranylpyrophosphate (GPP), a C_{20}-moiety, and cholesterol. FPP and GPP are also referred to as isoprenoids. They are essential for the activation of a variety of intracellular proteins. In this process, called (iso)prenylation, farnesyl or geranylgeranyl-moieties are coupled to the protein, resulting in a farnesylated or geranylgeranylated protein. These reactions are catalyzed by farnesyltransferase and geranylgeranyl-transferase, respectively. Several proteins involved in signaling are dependent on prenylation for their activity, such as ras, rho, nuclear lamins, transducin c, rhodopsin kinase, and G proteins. Consequently, statins lead to pleiotropic effects. [59]

Several of the proteins dependent on posttranslational prenylation, either farnesylation or geranylgeranylation, such as ras, rhoA and rhoC, have been linked to cancer pathogenesis. For example, *ras* is a known oncogene and ~30% of human tumors harbor *ras* mutations resulting in aberrant ras activity which is dependent on prenylation. [59] Specifically, N-*ras* and B-*raf* mutations are observed in ~30% and ~60% of melanomas, respectively. [60] N-*ras* and B-*raf* mutations both result in activation of the so-called Ras/Raf/MEK/ERK signaling pathway. [13] Raf which is downstream of ras, however, does not require prenylation to achieve full biological activity. [61] Still, in melanomas with a B-*raf* mutation, but no *ras* mutation, possible antineoplastic effects may be mediated through for instance rhoA or rhoC. Potential chemopreventive agents that may interfere in this pathway are: statins, FTIs, GGTIs, apomine, and perillyl alcohol. [13;59]

Furthermore, the rho family is involved in signaling and regulation of cell differentiation and proliferation. [62;63] Moreover, high-throughput screens for transcriptionally regulated targets involved in metastasis have shown that rhoC overexpression is strongly associated with the metastatic potential of inoculated melanoma in mice. [64] Indeed, *in vitro* and animal melanoma studies show a potentially chemopreventive activity of statins. More specifically, anti-tumor effects exerted by statins have been shown to include: 1) inhibition of tumor growth, 2) induction of apoptosis, 3) reduce invasiveness and metastasis, and 4) effects on angiogenesis.

Ad 1. Lovastatin, mevastatin, and simvastatin, but not pravastatin, reduced tumor growth of human melanoma cell lines HT144, M14, and SK-MEL-28 *in vitro* with IC_{50} values between 0.8 and 2.1 μM. [65]

Ad 2. Jani *et al.* observed induction of apoptosis by lovastatin in murine B16F10 melanoma cells through a geranylation-specific mechanism [66]; Additionally, increased apoptosis, in a dose-dependent manner, was observed in human M14 cells after 72-h incubations (4-8 μM) of lovastatin, mevastatin, and simvastatin. [65] In human A375 melanoma cells, Shellman *et al.* also showed induced apoptosis by lovastatin. [67] Interestingly, Shellman and colleagues also performed add back experiments showing that supplementation of GPP, but not FPP, blocked the apoptotic effect of lovastatin which indicates apoptosis must involve proteins dependent on geranylgeranylation. [67]

Ad 3. Atorvastatin (1-3 μM) reduced invasiveness of A375M, CHL, SK-MEL-28 and WM 166-4 melanoma cells in an experiment performed by Collisson and colleagues. *[68]* In this experiment, atorvastatin (4 dd 10 mg/kg orally also reduced metastasis of A375M melanocytes in severe combined immunodeficient (SCID) mice. [68] Likewise, Jani *et al.* showed reduced metastasis by lovastatin and simvastatin in murine B16F10 melanoma cells. [66] Experiments reported by Glynn *et al.* also showed decreased invasiveness by lovastatin, mevastatin, and simvastatin on HT144, M14, and SK-MEL-28 cells. [65]

Ad 4. Lovastatin (2-12.5 μM) exhibited a concentration-dependent pro-angiogenic influence on A375M and G361 cells in an angiogenesis model with a co-culture of HUVEC cells (human umbilical vein endothelial cells) and human diploid fibroblasts (HDF). [69] However, in nonmelanoma cells, some studies with low-dosed statins have suggested increased angiogenesis. [59]

Some statin-mediated effects appear to be completely independent of HMG-CoA reductase and cholesterol lowering. E.g., some experiments with statins in the closed ring form, which do not inhibit HMG-CoA reductase, do show *in vitro* anticancer effects. [70] Further investigations on these cholesterol-independent pathways are needed.

Examples of the cholesterol-independent pathways that have been suggested are:

- binding to the leukocyte function antigen-1 (LFA1) which has an important role in leukocyte migration and T-cell activation. [71]
- inhibition of the proteasome [70;72;73] which could for instance account for effects on the cyclin-dependent kinase inhibitors (CDKIs) p21 and p27 [74], and increased fibrinolytic activity [75].
- altered membrane receptor function due to changes in membrane fluidity caused by cholesterol depletion. For example, melanocortin receptor (MC1R) [76] or

insulin-like growth factor receptor function [77-79], both of which are involved in melanocyte and melanoma growth.

In addition, some investigators suggest direct toxic effects of cholesterol lowering are involved. [80] Malignant cells metabolize cholesterol differently and, therefore, may be more sensitive. However, the evidence for this hypothesis is (very) limited.

Although *in vitro* and animal experiments in general show promising results, some critical issues should be mentioned. E.g., pravastatin, the only hydrophilic statin, does not exhibit clear chemopreventive effects in most experiments. Moreover, most studies have used statins at serum concentrations and dosages that exceed doses applied for the treatment of hypercholesterolemia. Lovastatin dosed at ~1 mg/kg/day, for example, yields steady-state serum concentrations of 0.15–0.3 μM. [81] Often tumor cell lines were only sensitive to lovastatin at higher concentrations, e.g. 1.0-12.5 μM. [65;67;69]

Interestingly, some agents may have synergistic chemopreventive action together with statins. For example, d-γ-tocotrienol (5 μM) together with lovastatin (1 μM) totally blocked cell growth, whereas lovastatin (12%) and d-γ-tocotrienol (8%) individually showed only limited growth inhibition in these concentrations. [82] Other agents that have been suggested in combination with statins are NSAIDs, bisphosphonates, GGTIs, phosphoinositide 3-kinase (PI3K) inhibitors, CDKI, MEK inhibitors, and tyrosine kinase inhibitors. [59]

Evidence for efficacy in humans

Originally, RCTs testing statins for cardiovascular disease were the first to report on a possible decreased cancer incidence with statin use. [56] Ironically, concerns about increased cancer incidence with low cholesterol led to inclusion of cancer as a secondary safety outcome in these trials. Since then, a large number of meta-analyses and observational studies investigating statin use and cancer incidence were performed.

Additionally, two abstracts appeared on a preliminary case control study comparing the use of statins among 74 melanoma cases and age, gender and race-matched controls. Preliminary results in this study were promising (OR = 0.55, $p = 0.11$). [83;84] However, to the best of our knowledge, the results of the final analysis have not been published.

Shortly after these reports, two large population-based studies reported decreased incidences of cancer. [85;86] Our group performed a large observational study (3129 statin users & 16976 nnon-users) in which statin use was associated with a 20%

decrease in cancer incidence (OR = 0.80, 95% CI = 0.66-0.96). The association was more pronounced with prolonged use (statin use ≥ 4 yrs, OR = 0.64, 95% CI = 0.44-0.93). [85] Subsequently, Poynter and colleagues reported, among 1953 patients with colorectal cancer and 2015 controls, a significantly reduced risk of colorectal cancer (OR = 0.50, 95% CI = 0.40-0.63) with the use of statins (≥ 5 years versus nonusers). [86] However, since then, research has shown conflicting, and generally disappointing results for statin use as a general cancer chemopreventive agents. [87-89] Moreover, some meta-analyses suggest differences in the associations between statin use and incident cases of different cancer types. [89]

Dellavalle *et al.* performed a formal Cochrane review on specifically incident melanomas as a secondary outcome of RCTs with primary cardiovascular outcomes. In this Cochrane review, 6 statin RCTs providing data on incident melanomas were included. Overall, 59 melanomas occurred among the participants randomized to statin treatment and 67 incident melanomas occurred in the placebo groups. The resulting odds ratio was 0.90 (95% CI = 0.56-1.44) indicating no statistically significant difference. However, due to the low numbers of incident melanomas, a (clinically relevant) association cannot be excluded. More importantly, three of the included RCTs studied pravastatin which may have, as *in vitro* studies have suggested, diminished chemopreventive activity. Interestingly, a subgroup analysis by type of statin showed a reduced melanoma incidence for lovastatin (OR = 0.52, 95% CI = 0.27-0.99). This analysis is, however, importantly limited by the fact that there was only one trial with lovastatin. The authors' final conclusions were "… does not exclude the possibility that these drugs (i.e., statins and fibrates) prevent melanoma …". [90]

Additional RCTs have been published since the Cochrane review. In a meta-analysis published in The Lancet, the Cholesterol Treatment Trialists' (CTT) Collaborators included 14 RCTs of statins and found no evidence for a decreased cancer incidence (RR = 1.00, 95% CI = 0.95-1.06). In a sub analysis among the trials for which melanoma incidence was available, there was also no statistically significant change in melanoma incidence (RR = 1.03, 95% CI = 0.71-1.50). [88] Another six similar meta-analyses have reported on melanoma incidence with estimates for melanoma incidence ranging from 0.84 to 1.5. [87;89;91-93] However, they mainly included the same RCTs.

Table 2 presents an overview of RCTs in cardiovascular disease comparing statins with placebo, no treatment or usual care and from which melanoma incidence was reported.

These clinical trials, however, have several disadvantages which include small numbers of incident melanomas, relatively short follow-up for melanoma incidence (ranging from 3 to 6 years) and, generally, of being a retrospective reviews of cardiovascular

trials in which the design was not adapted for the analysis for melanoma incidence. For instance, they would not be stratified for factors, we would recognize now as critical to melanoma development, such as the family history of melanoma, skin type, presence or absence of clinically atypical nevi et cetera. Therefore, retrospective analyses on these trials will always be of limited value.

The number of epidemiological studies reporting on the potential association between incident melanomas and statin use is very limited. Kaye and Jick reported a case-control study on cancer and statin use that performed in the GPRD (General Practitioners' Research Database) in the UK. In a sub analysis within this study, they observed a relative risk of 2.5 (95% CI = 0.78-7.3) among 79 incident melanoma cases between 1990 and 2002 and up to five controls matched on year of birth, sex, general practice, year of entry into the GPRD, and index date. The follow-up in this study ranged between 3 and 13.7 years with a median of 6.4 years. [94] However, the number of melanoma cases in this study was relatively small as reflected in the wide confidence interval.

In a larger case-control study, we also reported on statin use and melanoma incidence. In this study, we used data from the Dutch national pathological database and from PHARMO, a pharmacy database covering ~25% of the Netherlands. Among 1,318 melanoma cases (primary diagnosis 1991-2004) and 6,786 controls matched on gender, date of birth and geographic region, we could not validate an association between statin use (≥½ y) and melanoma incidence (OR = 0.98, 95% CI = 0.78-1.2). However, the Breslow's depth of the melanomas was reduced among statin users (–19%, 95% CI = –33% to –2.3%). In a pre-specified stratified analysis across gender, we observed that the difference was nonsignificant among women (–4.8%, 95% CI = –29.6% to 28.8%), and more pronounced in men only (–27.8%, 95% CI = –43.7% to –7.4%). The lack of an association on melanoma incidence in our study could be due to the relative short follow-up which was, by design, was 3 years for all individuals. {Koomen, 2007 1003 /id}

Noteworthy, in the PRIME study, a prospective cohort study, Gardette *et al.* recently observed a reduced cancer mortality, although statistically non-significant, among dyslipidemic men using statins as compared to untreated dyslipidemic men (OR = 0.41, 95% CI = 0.19-1.06). [96]

These observational studies, however, have the disadvantage of being non-randomized and observational for which (residual) confounding cannot be excluded. Moreover, risk factors critical to melanoma development, such as the family history of melanoma, skin type, presence or absence of clinically atypical nevi et cetera, will often not be available for adjustment in the analyses. If so, confounding may have resulted.

In summary, results of secondary analyses of cardiovascular trials and of observational research on the potential relation between statin use and incident melanomas are conflicting. Both these RCTs as well as the epidemiological studies have some important limitations such as potential residual confounding, and small numbers of incident melanomas and thus limited power. Therefore, efficacy of statins in melanoma chemoprevention can neither be validated nor excluded.

Safety, Tolerability & Compliance

In cancer chemoprevention literature, the excellent safety profile of statins in cardiovascular disease has often been pointed out. [12-14;97] Indeed, statins have relatively mild side effects in the doses used to prevent cardiovascular event. The most prominent side effects of statins are the so-called statin-related myopathy (i.e., muscle pain and weakness), elevated creatinine kinase (CK) levels and as a rare but life-threatening side effect, rhabdomyolysis. In RCTs the incidence of myopathy was 1.5-5%, whereas estimates in observational research indicated 5-10%. [98] In spite of the fact that the majority of side effects are mild, persistence to statins in the use for cardiovascular disease is poor with only ~25% of patients still compliant 5 years after starting statin therapy. [99] To ensure compliance and persistence, an excellent tolerability is needed.

In cancer chemoprevention, higher day doses may be required. In such high doses, the tolerability of statins has been proven to be limited due to dose-dependent side effects such as myopathy. In phase I /II trials for cancer treatment significant responses were only achieved with >25 mg/kg/day doses leading to dose-limiting toxicities (DLTs) including myalgia, muscle weakness, elevated CK activity, anorexia, ulcerative lesions, rhabdomyolysis, nausea, diarrhea, and fatigue. With very high statin doses, cardiomyopathy may even be a side effect. [100] In the trials mentioned, among others cycled dosing with 3-4 week intervals was introduced to prevent DLTs. [81;101] For melanoma chemoprevention, it remains uncertain which doses are required. However, since cell lines studies often indicate cytostatic rather than cytotoxic effects at achievable *in vivo* statin concentrations, continuous dosing is likely to be required. [102]

Numerous risk factors for statin-related myopathy have been described. [98] Among these risk factors is using high statin doses which, as mentioned before, may be required for chemopreventive effects. Some of the risk factors may be circumventable, such as excessive physical activity, perioperative period and concomitant use of drugs or grapefruit juice which precipitate drug interactions associated with elevated serum statin levels. For atorvastatin, lovastatin, cerivastatin or simvastatin, these are CYP3A4 inhibitors and for fluvastatin these would be CYP2C9 inhibitors. [98] Avoiding the risk

factor, temporary cessation of statin therapy or drug alternatives for the inhibitors can be options in these cases. Non-preventable risk factors, such as advanced age, female gender, (relative) renal insufficiency, hypothyroidism, alcoholism or (family) history of myopathy or CK elevation [98], should be considered as special subgroups in formal risk-benefit analyses. Some of the non-preventable risk factors might be considered contraindications for statin therapy, e.g., (relative) renal insufficiency.

The causal mechanism of statin-related myopathy is not entirely unraveled. Among the proposed mechanism is depletion of ubiquinone (also referred to as coenzyme Q10). Ubiquinone, a side-product in the mevalonate pathway, is widely used as a non-drug 'over the counter' (OTC) anti-aging agent, but studies on its long-term safety are sparse. Concomitant use of ubiquinone may, however, prove to be a good candidate to increase statins' tolerability. Indeed, Thibault and colleagues have used adding Q10 to lovastatin therapy for doses 30 mg/kg/day as a strategy to prevent statin-related myopathy and increase tolerability. From these preliminary data, this strategy seems to be promising. [81]

Further research is needed to explore the precise mechanisms involved in statin-related myopathy and, after required statin doses have been established, to determine the long-term safety of this chemopreventive strategy.

In summary, long-term safety data for low dose statins is excellent, but may be less favorable for higher doses that are likely to be required for chemoprevention of melanoma. Development of a chemopreventive strategy including risk factors for statin-related myopathy and preventive measures may ameliorate the risk-benefit ratio.

Conclusion Statins

Statins inhibit HMG-CoA reductase leading to inhibition of isoprenylation of several proteins involved in melanoma development and progression, such as ras, rhoA and rhoC, and which are dependent on this posttranslational prenylation. HMG-CoA independent pathways may, however, also be involved. Experiments have shown anti-tumor effects of statins to include: 1) inhibition of tumor growth, 2) induction of apoptosis, 3) reduce invasiveness and metastasis, and 4) effects on angiogenesis. These *in vitro* and animal experiments show promising results. However, concentrations and dosages used in these experiments often exceed doses applied for the treatment of hypercholesterolemia. Additionally, chemopreventive activity may depend on which statin is used (e.g., lovastatin > pravastatin).

Up to now, the results of secondary analyses on cardiovascular trials and observational have been conflicting. Both study types have some important limitations, such as

such as lack of power, relatively short follow-up, low doses and imperfections in study designs. Thus, the promising results observed in preclinical experiments can neither be validated nor excluded.

Although, long-term safety data for low dose statins are excellent, they may be less favorable for higher doses that are likely to be required for melanoma chemoprevention. Development of a chemopreventive strategy including risk factors for statin-related myopathy and possible preventive measures, such as adding ubiquitinone to statin therapy, may ameliorate the risk-benefit ratio. First, however, efficacy in humans should be sufficiently proven.

Further studies on the involved pathways and possible cross links with other pathways, cholesterol-independent pathways, dependence of efficacy on melanoma mutational status, required dosages, possible differential effects between statins, and the temporal and dose-response cause effect relationships are required.

Fibrates

Fibrates are used as lipid-lowering therapy to prevent cardiovascular events. This drug class consists of bezafibrate, clofibrate, ciprofibrate, etofibrate, fenofibrate, gemfibrozil, simfibrate, and ronifibrate. The hypothesized mechanism by which fibrates alter lipid metabolism is thought to be peroxisome proliferators activated receptor-α (PPAR-α) agonism [80], which stimulates the oxidation of fatty acids.

Mechanism of action

The interest in a possible association between use of fibrates and cancer has been raised by three observations. First, ecological research showed an increased cancer incidence with low cholesterol. [57] Secondly, gemfibrozil promoted the development of liver cancer in rodents. [58] Thirdly, decreased cancer incidences have been reported in RCTs testing lipid-powering drugs for cardiovascular disease. [56]

The molecular mechanisms underlying potential chemopreventive properties of fibrates are not clearly defined. Several mechanisms have been hypothesized. For example, some authors believe that direct toxic effects of cholesterol lowering on melanoma cells may be responsible. In explanation, cholesterol lowering may have differential effects in malignant cells and normal cells because cancerous cells metabolize cholesterol differently. [80] The possible relationship between cholesterol and cancer are, however, poorly understood.

An alternative hypothesis concerns PPAR-α or PPAR-γ agonism by fibrates which is assumed to mediate growth inhibition and apoptosis. [103-105] Grabacka and colleagues demonstrated inhibition of migration by fenofibrate in a murine B16F10

and a human SkMell88 melanoma cell line. These effects were reversed by a PPAR inhibitor. The authors suggested PPAR-α is involved. However, in an *in vitro* study of Mössner *et al*. PPAR-γ specific agonists, such as rosiglitazone, inhibited cell proliferation in four melanoma cell lines dose-dependently, whereas a specific agonist of PPAR-α receptor had no such effect. [104] Therefore, some researchers believe PPAR-γ agonism is involved in the chemopreventive effects of fibrates on melanoma. To test the hypothesis that PPAR-γ is important for the risk of melanoma development, Mössner and colleagues also investigated the possibility that variations in the gene encoding PPAR-γ influence melanoma risk. In two independent case-control studies with in total 832 melanoma cases and 790 controls, they studied two gene variants (P12A[rs1801282] and C161T [rs3856806]). In one study, cases, compared to controls, were more likely to be a homozygous carrier of a *T allele of the C161T polymorphism in exon 6 of PPAR-γ (6.0 versus 2.0%; p <0.01). After adjusting for melanoma risk factors, such as skin type and nevus count, the association was still significant (OR = 5.2, 95% CI = 1.7-16.0). In the second case-control study, however, this finding could not be replicated. They finally concluded that the investigated PPAR-γ polymorphisms are not likely to constitute a significant risk factor for melanoma risk among German Caucasians. [106] These conflicting results, however, warrant further study.

Alongside with growth inhibition and apoptosis, fibrates may also have antimetastatic effects. Grabacka *et al.* showed that hamsters with allograft melanoma cells and treated with oral fenofibrate developed significantly fewer metastatic lung foci compared to controls. [107]

Evidence for efficacy in humans

In the Cochrane review by Dellavalle and colleagues, seven fibrate trials provided data on incident melanomas. In five of these RCTs, incident melanomas were diagnosed. Although there was an overall 42% reduction in melanoma incidence with use of fibric-acid derivatives (OR = 0.58, 95% CI = 0.19-1.82), this reduction was not statistically significant. Subgroup analyses by gender, trial funding, or type of fibrate, failed to show statistically significant differences in melanoma outcomes. [90] The value of these subgroup analyses is, however, limited due to small numbers.

In a meta-analysis that also included RCTs with a shorter duration (≥ ½ year in stead of ≥ 4 years), Freeman *et al.*, reported an overall odds ratio of 0.45 (95% CI = 0.20-1.01). [92] Most of the included trials, however, were also included in the Cochrane review. Additionally, for these clinical trials several disadvantages apply which were mentioned earlier (see statins – efficacy in humans).

To our knowledge, since the Cochrane review, no additional cardiovascular RCTs

studying fibrates have been published that reported the number of incident melanomas.

Some observational studies have focused on fibric-acid derivatives and cancer incidence. For instance, Poynter *et al.* published a case-control study among 1953 cases with colorectal cancer and 2015 controls. However, in this study, cases did not use fibrates more often than controls (OR = 1.08, 95% CI = 0.59-2.01). [86]

Epidemiological studies on fibrates and, specifically, melanoma incidence are thus far not available. Some epidemiological studies on statins and cancer or melanoma did, however, include a drug group of 'other lipid-lowering drugs' but this also includes bile acid-binding resins and nicotinic acid and its derivatives. {Graaf, 2004 1398 /id;Koomen, 2007 1003 /id} Moreover, recently Gardette and colleagues demonstrated in the PRIME study that cancer mortality among dyslipidemic men using fibrates is about half the cancer mortality among untreated dyslipidemic men (OR = 0.52, 95% CI = 0.28-0.97). [96]

In conclusion, although secondary analyses of cardiovascular trials with fibric-acid derivatives in two available meta-analyses have been promising, data from observational research or new clinical trials are largely lacking. The lack of such new subsequent studies is likely to be a reflection of the diminished interest in fibrates as lipid-lowering therapy.

Safety, Tolerability & Compliance

Over the last four decades, both clinical experience and large long-term RCTs in the cardiovascular setting have provided safety data on gemfibrozil, fenofibrate, bezafibrate, and ciprofibrate. Side effects related to fibric-acid derivatives include abdominal pain, dyspepsia, myopathy, myalgia, elevated CK levels, rhabdomyolysis, reversible increases in serum creatinine and urea, and cholelithiasis. Venous thrombosis, pulmonary emboli, and increases in homocysteine levels (clinical relevance uncertain) have also been reported. [108]

Myopathy, myalgia, elevated CK levels, and rhabdomyolysis are consistently reported with the use of fibric-acid derivatives, both in monotherapy as well as in combination with statins. Although rare, these side effects, especially rhabdomyolysis, are among the most serious safety risks of fibrate exposure. Both rhabdomyolysis and other muscle symptoms occur more frequently with gemfibrozil (~3.7 per 10,000 person years, 95% CI = 0.8-11) than with fenofibrate (~0 per 10,000 person years, 95% CI = 0-15). The mechanism of fibrate-related myotoxicity is not entirely unraveled, but the risk seems to be increased for patients with diabetes, renal failure, advanced age, hypothyroidism, and most importantly with concomitant use of statins. [108] Notorious

is the concomitant use of gemfibrozil with cerivastatin or fluvastatin. Gemfibrozil precipitates a drug-drug interaction leading to increased exposure of these statins metabolized via CYP2C8/9, which in turn has been shown to be related to an incidence rate of rhabdomyolysis of ~1,000 per 10,000 person years. [108] Due to reports of rhabdomyolysis, with concomitant use of gemfibrozil, cerivastatin was withdrawn from the market in 2001.

Increases in serum creatinine levels have been observed with fenofibrate, bezafibrate, ciprofibrate, and, less commonly, gemfibrozil. Both an increased production of creatinine as well as a reversible decrease in glomerular filtration rate (GFR) has been postulated as the molecular mechanism behind this side effect. [108] Several studies, however, did not show decreased renal function nor an increased incidence of renal failure. Moreover, in patients without impaired renal function, creatinine elevations are reversible upon discontinuation of the fibrate. In patients with preexistent renal dysfunction, however, fibrates should be used cautiously in adjusted doses. [108;109]

Fibrates appear to be lithogenic meaning that they increase the cholesterol saturation in the bile and may cause gallbladder disease. Risk factors for coronary artery disease are, however, also risk factors for gallbladder disease. Epidemiologic studies comparing the incidence of gallbladder disease with and without fibrate therapy are, therefore, likely to overestimate the incidence of this side effect. Nevertheless, this side effect has been validated with trial data [108] and should be considered a relatively rare but potentially serious side effect.

In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial, use of fenofibrate, compared to placebo, seemed to be associated with slight increases in the incidence of deep venous thrombosis (1.4 versus 1.0%), and pulmonary emboli (1.1 versus 0.7%). [109] Whether these findings indicate true side effects or if they are artifacts due to multiple simultaneous comparisons in this dataset remains under debate. [108;109]

A number of potential health benefits related to use of fibrates has been demonstrated or suggested. For example, clofibrate has been shown to reduce myocardial infarctions, for example in the Coronary Drug Project and a WHO trial. In this latter study, however, clofibrate, compared to placebo, was reported to be associated with a significant increase in overall mortality. Subsequent analyses have demonstrated that the increase was artificially caused by the study design which resulted in a biased follow-up of the participants randomized to clofibrate. [109] Nevertheless, analyses of cardiovascular, cancer-related and overall mortality within the target population should be part of any chemoprevention trials since these would be essential to assess the overall risk-benefit balance.

An additional potential health benefit was observed in the FIELD trial. Among diabetes patients, less progression of albuminuria was observed with fenofibrate use. [110] Within the Diabetes Atherosclerosis Intervention Study (DAIS) a reduction in proteinuria in the fenofibrate group was reported as well. [109]
Overall, the safety profile of fibrates is good if used for lipid-lowering as an alternative for, or additional to, statins. However, the required doses of fibrates as a melanoma chemo-preventive drug are unclear and long-term data on overall mortality rates and rare side effects are limited. These data would be essential for formal risk-benefit ratio analyses.

Conclusion Fibrates

Despite the promising results in two meta-analyses, the evidence for efficacy of fibrates in melanoma or cancer chemoprevention is inconclusive. Additionally, a valid molecular mechanism for the antineoplastic effects of fibric-acid derivatives has not been sufficiently described so far. Thus, further research on the molecular mechanisms behind and required dosing for the potential chemopreventive effects of fibrates on melanoma is warranted and the efficacy of fibrates in melanoma chemoprevention cannot be validated yet. Subsequently, long-term safety and mortality data would be required to assess the risk-benefit balance for melanoma chemopreventive strategies which include the use of fibrates.

Retinoids (Vitamin A and derivatives)

The group of the so-called retinoids includes vitamin A and its derivatives. Analogs are either naturally occurring or synthetically derived. First generation retinoids include vitamin A (all-*trans* retinol), tretinoin (all-*trans* retinoic acid), and isotretinoin (9-*cis* retinoic acid). Acitretin and etretinate belong to the 2nd generation retinoids, whereas adapalene, bexarotene, and tazarotene are examples of 3rd generation retinoids.
Retinoids are in use as acne treatment or anti-aging agent, but may also be used for several other indications, such as acute promyelogenous leukemia (APL). [80;111] Natural retinoids are also present in dietary sources, and are involved in several physiological processes among which vision, embryonic development, and regulation of growth and cell differentiation. [112]

Mechanism of action

Retinoids are thought to exert most of their effects by binding to retinoid acid receptors (RAR) and retinoid X receptors (RXR) in the cellular nuclei leading to altered gene transcription. [112;113] Different genes encode the α, β, and γ receptors which in

turn have two (RAR-α, RAR-γ) or four (RAR-β) splice variants. Tretinoin binds and activates only the RAR receptors, whereas isotretinoin is both a RAR and RXR agonist. [112] Rexinoids, 3rd generation retinoids, selectively bind to RXR which is hypothesized to be especially involved in proapoptotic effects. One of these agents, bexarotene, has been approved by the FDA for cutaneous T-cell lymphoma. [13] Because melanoma is known to be relatively resistant to apoptosis, retinoids and rexinoids, in particular, may also be interesting candidates for melanoma chemoprevention.

Chemopreventive effects exerted by retinoids/rexinoids may include: 1) inhibition of tumor growth, 2) promotes cell differentiation, 3) induction of apoptosis, 4) proangiogenetic effects, and 5) reduced invasiveness and metastasis.

Ad 1. Tretinoin markedly reduced cell growth of B16 murine melanoma cells at a concentration of 10^{-7} M. [114] Additionally, mice treated with vitamin A before being inoculated with murine melanoma cells had significantly decreased tumor growth compared with controls. [115] Moreover, CD437, a synthetic RAR-γ selective retinoid, inhibited the cell growth *in vitro* of three human melanoma cell lines (MeWo, SK-Mel23, and MV3) in a concentration-dependent matter (IC_{50} value: 5×10^{-6} M), whereas tretinoin did not. In the same study, CD437 was shown to decrease tumor volume in a xenograft MeWo mouse model. [116]

Ad 2. Retinoids have also been shown to promote cell differentiation of the mouse B16 melanoma cell line. [111]

Ad 3. CD437 was observed to induce apoptosis in MeWo melanoma cells *in vitro* after 72 h incubation at a concentration of 5×10^{-6} M. [116] Likewise, in another study, CD437 also promoted marked apoptosis in A375 melanoma cells at this concentration. [117]

Ad 4. Tosetti *et al*. postulated additional antiangiogenic effects of retinoids since tretinoin has shown antiangiogenic effects in several systems. [118] although antiangiogenesis was demonstrated in other tumor types, it has not been demonstrated (yet) for melanoma.

Ad 5. In an experiment by Edward and colleagues, pretreatment with 10^{-6} M tretinoin of metastatic B16 melanoma cells resulted in a significant inhibition of lung colonization after injection of 10^5 cells into the tail vein of mice. [119]

Although RAR and RXR receptors are generally thought to be involved in these chemopreventive effects, the exact mechanisms remain unclear. Moreover, studies with synthetic retinoids have revealed that apoptosis and growth inhibition mediated by these agents are likely to be independent of this retinoid signaling pathway. [120;121] These RAR/RXR independent pathways are supported by several observations:

- apoptosis could be induced in tretinoin-resistant cells.

- retinoid receptor antagonists failed to inhibit apoptosis induced by synthetic retinoids.
- retinoid related molecules that do not bind to retinoid receptors can be effective inducers of apoptosis. [121]

Alternative mechanisms that may be involved are inhibition of mitogen-induced c-*fos* expression [114], NF-κB activation mediated by retinoid acid inducible gene I through a CARD-containing adaptor protein VISA [117], and enhanced production of reactive oxygen species (ROS) dependent on Rac activity [122]. Examples of additional hypothesized signaling pathways include increased expression of p16, p21, p27, p53, and bax, decreased expression of Id1 protein, and down-regulation of mitogen-activated protein kinase and bcl-2. [80]

Overall, *in vitro* studies of murine and melanoma cell lines have produced some evidence for chemopreventive effect of retinoids and rexinoids on melanoma. However, the evidence as yet is not well enough established and the involved mechanisms are not distinctly defined.

Evidence for efficacy in humans

Anticancer effects of retinoids in certain types of human cancers are well-established. For instance, tretinoin (Vesanoid®) is used in the treatment of APL and has been approved by the FDA for this indication. In addition, high-dose isotretinoin has been successfully used in the chemoprevention of nonmelanoma skin cancer (NMSC) in patients with xeroderma pigmentosum. It reduced the incidence of NMSC by 63%. [123] The evidence for a role of retinoids in melanoma chemoprevention is, however, preliminary. Studies on the dietary intake of vitamin A have shown promising results. In a case control study among 542 melanoma cases and 538 controls, Naldi *et al.* reported an OR of 0.57 (95% CI = 0.39-0.83) for the highest quartile of retinol intake versus the lowest quartile. [124] Similarly, Feskanich and colleagues, in a cohort study among 162,000 Caucasian US women, observed a relative risk ratio for incident melanoma of 0.39 (95% CI = 0.22-0.71) for consumption of ≥1800 mcg/day of retinol as compared to <400 mcg/day (*p*-linear trend = 0.01). [125] Strong correlation between different food items and food groups as well as between diet and other health behaviors, however, dramatically complicate the interpretation of such nutritional and observational studies.

To our knowledge, there are no studies evaluating the effect of retinoids on melanoma incidence in humans. Despite this lack of definite data, a number of studies have evaluated the effect of topically or orally applied retinoids on surrogate markers lesions of melanoma, dysplastic or atypical nevi. Originally, Meyskens and colleagues

performed two case series with topical tretinoin and oral isotretinoin, respectively, for patients with dysplastic nevi. Only 3 and 8 patients, respectively, completed the study. Importantly, these studies did not include a control treatment. [126;127]
Edwards and Jaffe reported a preliminary randomized double-blind trial in which they randomized 21 patients with multiple large dysplastic nevi to either 0.05% tretinoin or placebo solution, both topically. Of the 8 patients randomized to tretinoin, 3 discontinued the study. Two of these patients discontinued due to local irritation. Seven of the 15 dysplastic nevi that were treated with tretinoin had completely disappeared or had reverted to normal, benign nevocellular nevi. [128] However, the small number of patients and the large proportion of drop-outs in the tretinoin group preclude definite conclusions. [128]
Halpern *et al.*, in a more recent trial, studied the effect of topical treatment with once daily 0.05% tretinoin or, if tolerated, twice daily 0.1% tretinoin for 6 months versus no treatment. An effect was observed on transformation of clinical appearance (including color, size, and border irregularities), and likewise, a statistically significant was shown on histological change toward benignity (for cellularity, cellular atypia, and proliferative cellular nuclear antigen). [129] Correspondingly, Stam-Postuma and colleagues evaluated topical treatment for 4 months with either 0.1% topical tretinoin, 0.1% tretinoin plus 1% hydrocortisone, or placebo cream. In their study, topical tretinoin 0.1% showed only clinical improvement with no improvement in the degree of atypia, possibly due to the limited number of biopsies. {Stam-Posthuma, 1998 1407 /id}
Due to the lack of validation of the predictive value of dysplastic nevi as a predictor of future incident invasive melanomas, the interpretation of these surrogate marker studies remains uncertain. As an additional limitation, these studies used different definitions for 'dysplastic nevi'. Noteworthy, toxicity has been substantial in these studies as indicated by the large proportion of drop outs and the high rate of patients experiencing side effects. Interestingly, some authors reported reappearance of a dysplastic nevus 1 year after cessation of topical tretinoin therapy. (128 and Stam-Postuma *et al.*, verbal communication)

Safety, Tolerability & Compliance

Retinoids' side effects include skin irritation following topical treatment and cheilitis (lip inflammation), xerosis, ocular effects, hepatotoxicity, hair loss, teratogenicity, bone toxicity, and serum lipid abnormalities following oral treatment. [80] Dose-dependent mucocutaneous irritation affects nearly all patients and is often the dose limiting side effect [113], but it is, in many patients, a temporary side effect [129].
From a doctor's point a view topical treatment may be preferred since it involves less

(serious) side effects. However, the use of topical retinoids in skin cancer chemoprevention trials, for example for patients with dysplastic nevi or in transplant patients, has been restricted by the irritation they cause. New, less irritating, formulations could be of interest. However, adherence to the application regimen with topical treatment may prove to be too big a hurdle for the use of topical retinoids in melanoma chemoprevention. Systemic retinoid therapy on the other hand has been associated with substantial toxicity [80] and thus may also lead to relatively rates of discontinuation.
Another concern, is the teratogenicity of retinoids. For example, isotretinoin exposure during pregnancy may cause craniofacial, cardiac, thymic and central nervous system (CNS) defects in about 30% of the developing fetuses. [131] Among children born without anatomical defects, an increased incidence of developmental delays and other CNS effects has been observed. Preventing fetal exposures has proven to be a difficult task requiring comprehensive risk management programmes. [131] After discontinuation of retinoid treatment pregnancy should be avoided until the drug is essentially cleared from the body. For some retinoids, such as etretinate and acitretin, this period is up to 2 years. This feature excludes its use as a chemopreventive agent among women of childbearing age. Retinoids should therefore only be considered for high risk target populations that would exclude women under the age of 45.

Conclusion Retinoids

Although retinoids have been considered a candidate for melanoma chemoprevention over the last decades, data on the efficacy in humans are still largely lacking. Evidence from experimental research is also inconclusive. Moreover, teratogenicity and limited tolerability lead to concerns whether retinoids as a monotherapy could be suitable as a melanoma chemopreventive strategy. Research should, therefore, focus on possible synergistic combinations with other chemopreventive agents.

Imiquimod and analogs

Imiquimod is prescribed and approved by the FDA for the treatment of external genital and perianal warts (caused by human papilloma virus), multiple actinic keratoses and superficial basal cell carcinomas. [13] It is an immune modifier that stimulates the immune system through Toll-like receptors, particularly TLR-7. [12] Imiquimod has been shown to induce apoptosis and, therefore, has also generated interest as a topically applied potential chemoprevention agent. [132]

Mechanism of action

The pivotal mechanism of action of imiquimod is stimulation toll-like receptors (mainly

TRL7) on dendritic cells, B cells and plasmacytoid cells which triggers a T helper cell type 1 (Th1) immune response and induces transcription of Th1 cytokines, such as interferon-α (IFN-α), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-12. [13;132-134] In this way, imiquimod activates mature dendritic cells after binding to TRL7 and activation signals will be sent to the T cells with the aid of co-stimulatory molecules such as CD40, CD80 and CD86. [132] Consequently, the Th1 immune response results in the activation of naïve T cells to transform into antigen-specific T cells directed against antigens expressed on condylomata, basal cell carcinomas and other potentially immunogenic skin lesions. [132]

Until recently, experiments with imiquimod did not focus on possible chemopreventive effects towards cutaneous melanoma. However, recently, some preliminary evidence was generated by Schön and colleagues. They performed experiments to test for effects of imiquimod and resimiquimod on apoptosis and also possible direct toxic effects. No direct toxic effects were observed on four different melanoma cell lines (Mel-HO, Mel-2A, A375, and MeWo) and normal human melanocytes (established from five different donors). Thus, they did not observe direct cytotoxicity. However, marked concentration-dependent pro-apoptotic effects on the Mel-HO and A375 melanoma cell lines were demonstrated with imiquimod concentrations ranging from 5 to 50 μg/ml. Normal melanocytes, Mel-2A or MeWo melanoma cells showed markedly weaker, if detectable at all, induction of apoptosis with imiquimod. In contrast, resiquimod, an analog of imiquimod, did not induce apoptosis in either of the cell lines studied. [133]

Evidence for efficacy in humans

Cancer chemopreventive effects of imiquimod have been observed in several settings, mainly involving (precursor lesions) of skin cancer. For example, phase II RCTs in which patients with actinic keratoses (AK), a premalignant condition that may progress to squamous cell carcinoma (SCC), were treated with 5% imiquimod three times per week topically, have shown statistically significant improvement in clinical and histological appearance, and the average number of AK. [132] Additionally, open label phase II studies have also demonstrated beneficial effects on superficial and nodular basal cell carcinoma (BCC). Similarly, preliminary studies have suggested regression after local application of 5% imiquimod cream for additional precursor lesions, such as Bowen's disease (SCC *in situ*), and vaginal intraepithelial neoplasia (VIN). [132] The evidence for melanoma chemoprevention specifically, however, is scarce.

In two case reports, regression of lentigo maligna (LM, melanoma *in situ*) lesions that could not be excised were observed. [132;135] Moreover, in a small case series of five

patients, Wolf *et al.* observed complete clearance of LM lesions after 13 weeks of application each night of 5% imiquimod cream. [136] We believe these results, although positive, should not be considered true melanoma chemoprevention because if left untreated not all LM lesions will progress to invasive lentigo maligna melanoma (LMM) and the latent period is estimated to be 10-50 years. {Stevenson, 2005 1510 /id} Likewise, in a case of disseminated cutaneous metastatic melanoma, local control of tumor growth has been observed after treatment with imiquimod three times per week for 18 weeks. [138] Although this may indicate that imiquimod could be beneficial for cutaneous metastatic melanoma if radiotherapy or surgery is impossible [138], if these results predict chemopreventive activity is uncertain.

No human studies, to our knowledge, have evaluated the effect of imiquimod on melanoma incidence. Thus, imiquimod has not yet been studied for true primary melanoma chemoprevention.

Nevertheless, human data on the effects of topical imiquimod on atypical nevi, surrogate markers lesions of melanoma are available. Somani and colleagues, in a small case series of three patients, evaluated the effect of imiquimod applied five nights per week for 12 weeks on a selected clinical atypical nevus. Imiquimod treatment failed to cause lesional resolution in these patients. [134] Likewise, Dusza *et al.* have studied topical imiquimod in a pilot study among 10 patients with atypical nevi and at least 8 large nevi (≥ 5 mm) on the trunk. Standardized photographs were compared at baseline and 4 weeks after completion of 16 weeks of imiquimod treatment (5% cream applied 3 times per week). In addition, histological assessment was performed of each patient's 4 largest study nevi. Size and morphology showed no obvious changes, but 4 of 14 treated nevi and 0 of 14 untreated nevi showed histological changes suggestive of partial regression ($p = 0.03$). [139]

Investigators of the University of Arizona are currently testing an analog of imiquimod among patients with dysplastic nevi. [12] This study may be an important step forward in unraveling the chemopreventive potential of imiquimod and its analogs.

In summary, some, but not all, of these preliminary studies have shown promising results. More importantly, definite data on melanoma incidence or validated precursors are lacking.

Safety, Tolerability & Compliance

In general, the side effects of topical imiquimod are mild to moderate. Side effects include local skin reactions (LSR), nausea, vomiting, headache, muscle weakness, fever, flu-like symptoms and fungal infection. [80]

LSR are most frequent, dose and frequency dependent and usually subside after a

resting period. Severe LSR usually are the DLT and some studies have reported that 16% of patients (4/25) required 4-week rest periods after a four-week treatment period with 5% imiquimod cream three times weekly. [132]
Although LSR are not considered to be severe medical conditions, they may have important implications for adherence in long-term therapy that would be required for melanoma chemoprevention.
Systemic side effects are rarely reported [132], but presumably are more likely to occur if large areas of the body would be treated or with application on areas with thin skin such as the face.
Since imiquimod treatment is often restricted to a duration of 6-16 weeks [132] , the long-term safety data required to evaluate the risk benefit ratio for melanoma chemoprevention are lacking.

Conclusion Imiquimod and analogs

Imiquimod, and possibly some of its analogs, can be considered candidates for melanoma chemoprevention. Thus far, however, data from *in vitro* and *in vivo* experiments as well as human efficacy data are scarce and inconclusive. Additionally, long-term safety data are lacking.

Acetaminophen

Acetaminophen is a frequently used analgesic and antipyretic drug that, in most countries, is available both as an OTC drug as well as on prescription. Acetaminophen is also referred to as paracetamol and has been demonstrated to be a selective COX-3 inhibitor. [140] Its anti-inflammatory action is relatively weak and therefore it is not considered to be a NSAID.

Mechanism of action

Experimental studies on acetaminophen's effects on melanoma murine models or cell lines are very limited. Recently, however, Vad and colleagues have reported on two such studies. They tested an acetaminophen concentration of 100 μM which showed considerable toxicity towards B16F0 and B16F10 murine melanoma cells and SK-MEL-28, MeWo, and SK-MEL-5 human melanoma cell lines, resulting in a loss of cell viability of 40 ± 3, 45 ± 7, 66 ± 8, and 60 ± 5%, respectively. No significant toxicity was observed in three nonmelanoma cell lines (BJ, Saos-2, PC-3). Thus, selective toxicity towards melanoma cells with an IC_{50} of ~100 μM was observed. Adding glutathione (GSH) prevented toxicity in SK-MEL-28 melanoma cells, whereas 1-bromoheptane, a GSH depleting agent, increased acetaminophen induced toxicity. Additionally,

acetaminophen led to ROS formation and mitochondrial toxicity in these cells. The authors suggest that tyrosinase plays a role in acetaminophen's toxicity and that acetaminophen is a tyrosinase substrate. [141]
In a second study, Vad *et al.* studied the *in vivo* efficacy and toxicity of acetaminophen in a B16F0 skin melanoma tumor model in mice. At acetaminophen doses of 60, 80, 100, and 300 mg/kg/day, from day 7 until 13 post melanoma cell inoculation, tumor growth inhibition by 7 ± 14, 30 ± 17, 45 ± 11 and 57 ± 3%, respectively, was demonstrated. If acetaminophen was dosed from day 1 through day 13, the inhibition was similar. [142]
Overall, these two studies show promising, but limited, evidence for chemopreventive activity of acetaminophen against melanoma.

Evidence for efficacy in humans

Human data on the effect of acetaminophen on melanoma are very limited as well. Interestingly, Wolchok *et al.* observed two partial responses in a phase I dose-escalation study among 27 patients with stage III/IV melanoma. In this study, patients received acetaminophen doses every 3 weeks (10, 15 or 20 g/m^2) combined with carmustine (BCNU, 10 to 150 mg/m^2), every other cycle. To prevent acetaminophen toxicity, 6-8 hours after acetaminophen infusion had stopped, N-acetylcysteine (NAC) was infused (loading dose of 140 mg/kg in 1 h with subsequently 17.5 mg/kg/h for at least 19 h or until acetaminophen levels had dropped below 20 mg/L). [143] Obviously, however, these results may simply reflect effect of carmustine and may not predict any chemopreventive potential.
Some epidemiological studies investigating NSAIDs and melanoma incidence have used acetaminophen as a comparison drug. For instance, Harris and colleagues reported that they did not observe an association between acetaminophen and the risk of malignant melanoma. In their case control study, among 110 women with melanoma and 609 controls, they observed an OR of 0.95 (95% CI = 0.45-1.98). [42]
Asgari and colleagues, in a large cohort study, also included exposure to acetaminophen in their cohort study in which they investigated the association between melanoma incidence and NSAID exposure. However, they did not report findings on the association between use of acetaminophen and incident melanoma. [47]
Friis *et al.* have also investigated the association between acetaminophen use and cancer (among which melanoma). In contrast with the studies previously mentioned, their interest was raised by concern about the carcinogenic potential of acetaminophen. This concern originates from the fact that phenacetin, the precursor of acetaminophen, was withdrawn from the market due to an established link with urinary tract tumors.
The standardized incidence rate (SIR) observed by Friis *et al.* in the total cohort of

acetaminophen users was 0.9 (95% CI = 0.6-1.2). After excluding patients with prescriptions of aspirin and other NSAIDs, the SIR was 0.6 (95% CI = 0.2-1.3). Thus, an association cannot be excluded nor confirmed based on these data. [144]

Safety, Tolerability & Compliance

In normal doses, acetaminophen only rarely causes side effects. However, when liver enzymes catalyzing the normal conjugation reactions are saturated, acetaminophen will be metabolized by mixed function oxidases. As a result, N-acetyl-*p*-benzoquinone-imine, a toxic metabolite, is formed which is inactivated by conjugation with GSH. If GSH is depleted, toxic effects on the liver and also in the kidney will occur. [145]
Side effects of acetaminophen are dermatologic and allergic reactions, such as urticarial rash or exanthema, hypothermia, and renal failure after chronic exposure. Among patients with Glucose-6-Phosphate Dehydrogenase (G-6-PD) deficiency, acetaminophen may cause anemia, hemolysis and methemoglobinemia. [145]
In doses just above the normal therapeutic doses, however, acetaminophen may cause liver failure. Patients with special risk factors, such as preexistent liver failure, exposure to CYP2E1 inducers, such as carbamazepine, isoniazide or barbiturates, or chronic alcohol exposure, have an increased risk of liver failure if exposed to acetaminophen overdose. Single acetaminophen overdose can be relatively safely treated with NAC infusion. Chronic acetaminophen overdose, however, cannot and often leads to the need for liver transplantation. [145] Therefore, if future experiments would demonstrate that high doses of acetaminophen are required for melanoma chemoprevention, safety aspects are likely to preclude its use as such.

Conclusion Acetaminophen

Preliminary promising results have been generated for acetaminophen in human melanoma cells, a murine melanoma model and in a phase I study treating phase III/IV melanoma patients (combined with carmustine). The first few epidemiological studies, however, have been disappointing. Acetaminophen doses in these studies may have been too low. In general, evidence for acetaminophen as a potential chemopreventive drug is inconclusive and very preliminary.

Dehydroepiandrosterone

Dehydroepiandroterone (DHEA) is a physiologic steroid that is produced in response to adrenocorticotropin (ACTH) stimulation by the adrenal gland. [146] Physiologically, DHEA is predominantly present as dehydroepiandrosterone sulfate (DHEAS), and is a precursor of androgens (e.g., testosterone) and estrogens [147], but other physiologic

roles of DHEA and dehydroepiandrosterone sulfate (DHEAS) have remained unclear. In many counties, DHEA is marketed as a dietary supplement and, therefore, are available in OTC formulations which do not require approval of the regulatory authorities, such as the FDA and European Medicines Agency (EMEA). Beneficial effects of DHEA have been claimed for numerous indications. For most of these, however, evidence is preliminary, if not lacking at all. One of the claims is chemopreventive potential toward cutaneous melanoma. [148]

Mechanism of action

A small number of experiments have investigated the effects of DHEA on melanoma. Richardson *et al.*, in an attempt to investigate why women have a survival benefit in metastatic melanoma, have performed *in vitro* experiments with DHEA. At a concentration of 1nM DHEA, they observed significantly enhanced invasion of A375 melanoma cells. In contrast, *in vitro* experiments by Kawai and colleagues, showed DHEA dose-dependently inhibited the growth of B16 mouse melanoma cells and enhanced melanin production, which may indicate induction of differentiation. [149] In conclusion, there is hardly any experimental evidence to support claims of chemopreventive activity of DHEA towards melanoma.

Evidence for efficacy in humans

To the best of our knowledge, only a single study investigated the association between DHEA and incident melanoma in humans. In a nested case-control study, the mean serum DHEA and DHEAS levels of 23 melanoma cases and 43 controls (matched for age, sex and race) were compared. No statistically significant differences in de DHEA(S) levels were detected between cases and controls. [148]

Safety, Tolerability & Compliance

In physiological doses DHEA is considered to be safe. However, good quality long-term safety data for higher doses are lacking.

Conclusion Dehydroepiandrosterone

Both experimental and human data on the chemopreventive potential of DHEA(S) have been disappointing. However, the number of studies that have been reported is small. Nevertheless, DHEA does not seem to be a good candidate as a melanoma chemopreventive drug.

Discussion

Initially, our literature search resulted in a large number of references. However, most of these had to be excluded and about 75% of the finally included references did not emerge from the systematic literature search. We believe this is a reflection of the fact that 'chemoprevention' is not defined as a MESH term. Research would certainly benefit from such a MESH term.

Although there was a large number of preclinical studies available for some candidate chemopreventive drugs, the interpretation remains troublesome. Particularly, preclinical *in vitro* and animal models usually have not been validated. Similarly, biomarkers and precursor lesions have also not been validated. Moreover, different definitions for precursor lesions, such as atypical / dysplastic, have been used in the present literature.

Additionally, experimental research usually includes one or two agents of a larger drug class. Some drug classes, such as NSAIDs, may, however, be chemically rather diverse. We believe experimental research should include at least one example of each chemical subclass. In explanation, what may be interpreted as lack of effectiveness of a complete drug class, could very well be a result of differential effects of different subclasses or even of individual agents. The same problem may arise in observational research. For example, the disappointing results for statins in observational research do not exclude differential effectiveness for lovastatin. Freeman and colleagues calculated that based upon the lovastatin subgroup analysis (which included only one trial), 244 people would need to be treated for 5 years to prevent one case of melanoma. Similar effectiveness (which cannot be assumed *a priori*) in a high risk population would decrease this number needed to treat and may even result in a realistic chemopreventive strategy.

Since the temporal dose-response and cause-effect relationships between the duration and dose of chemopreventive drugs and incident invasive melanoma are unknown, it is not clear which study design is to be preferred. Duration of drug use and also follow-up in many studies may have been too short and daily doses may not have been high enough.

For chemopreventive drugs to move forward from *in vitro* research, animal experiments and observational studies towards RCTs and ultimately clinical practice, overall acceptable risk-benefit ratio for the target population is to be expected. To achieve this, after efficacy has been proven, a *sine qua non* in this issue, full risk-benefit analyses should be performed to show the overall health impact for subpopulations at high risk of developing (a second) melanoma. Such risk-benefit analyses should take into account all important health

outcomes (Fig. 2). For example a risk-benefit analysis of aspirin should not only include cancer reductions in melanoma, but also in colorectal, esophagus, stomach, lung, breast, and ovarian cancer, as well as benefits on other health aspects, such as reductions of myocardial infarction, pulmonary embolism, and occlusive cerebrovascular events. In addition, risks of long-term aspirin treatment should include all important drug related adverse events, such as GI bleeds, ulcers, perforation, and haemorrhagic stroke. [55] However, the balance between health benefits and risks is complicated by several issues, such as the lack of clear-cut definitions for the target population to be treated, but also by age. Specifically, with increasing age not only do the absolute risks of cardiovascular events and GI bleeds increase, but simultaneously melanoma risks are changing. Lack of evidence on the temporal and dose-response cause-effect relationships even further complicate these issues since the expected prevalence of adverse effects depends on required dose and duration. Consequently, the influence of different chemopreventive strategies, varying in drug dose, duration, definition of the target population in order to include individuals at highest risk of cancer development and excluding individuals at highest risk of developing adverse events, with or without additional interventions to prevent adverse effects, and the age-specific changes in the risk–benefit ratio should be investigated. Recently, an international expert group, however, concluded that "gaps in our understanding of appropriate dose, duration, and age of use, do not support a formal risk–benefit analysis". [55]

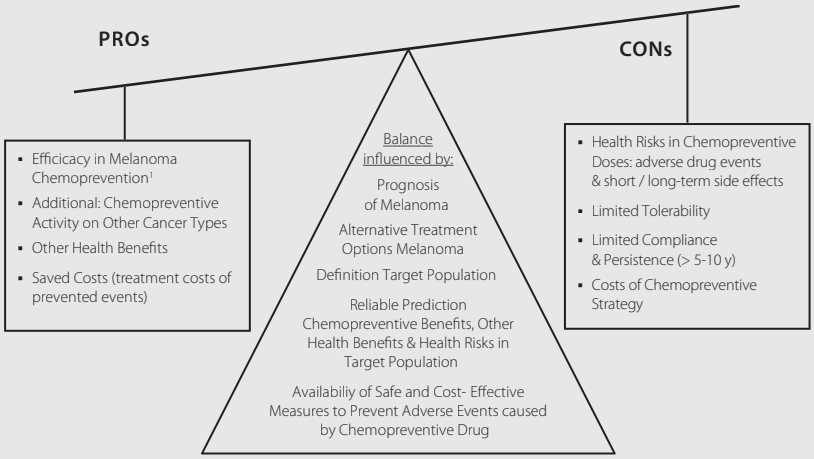
Nevertheless, among high risk (sub)populations, melanoma chemoprevention may prove to be an innovative approach additional to sun protection measures to control the increasing burden of melanoma in the future.

Conclusion

Considerable preclinical evidence of efficacy as a melanoma chemopreventive drug exists for aspirin, NSAIDs and statins. Data on clinical efficacy and long-term safety with doses required for melanoma chemoprevention, however, are still sparse. Validated preclinical models are urgently needed to move melanoma chemoprevention forward. In future research, special attention should be paid to explore possible differential effects within a drug class, temporal dose-response relationships, and to possible synergistic or antagonistic effects. Research should also focus on how to define the target populations.

Chemoprevention may prove to be an innovative approach additional to sun protection measures to control the increasing burden of melanoma among high risk

Figure 2 Risk-Benefit Balance of Melanoma Chemoprevention Strategies



[1] prevented or delayed incident melanomas including possible shifts in prognostic factors (e.g., distribution of Breslow depth, in situ/invasive, % ulcerated etc.) & inhibited progression of incident melanomas such as reduced tumor growth, invasiveness, metastasis.

individuals. Lack of definite data on efficacy in humans and profound long-term safety data in the required doses, however, preclude the use of chemopreventive drugs for melanoma in current practice. Success factors for melanoma chemoprevention to be useful in patient practice will likely be:

- Little-to-no toxicity, including mild but inconvenient side effects to not only ensure safety, but also tolerability and adherence
- A sufficiently motivated target population, e.g. patients with previous melanoma (or other types of cancer) or premalignant lesions would be more likely to be motivated to use a chemopreventive drug for at least 5 to 10 years.
- A clear-cut definition of the high risk subpopulations at whom chemoprevention should target based upon validated prediction models, mutational status and, if possible, validated early biomarkers of invasive melanoma risk
- A clear-cut definition of contraindications and predictors for individuals prone for the adverse events the chemopreventive drug may cause in order to withhold the drug from these individuals or to present additional preventive measure to them.

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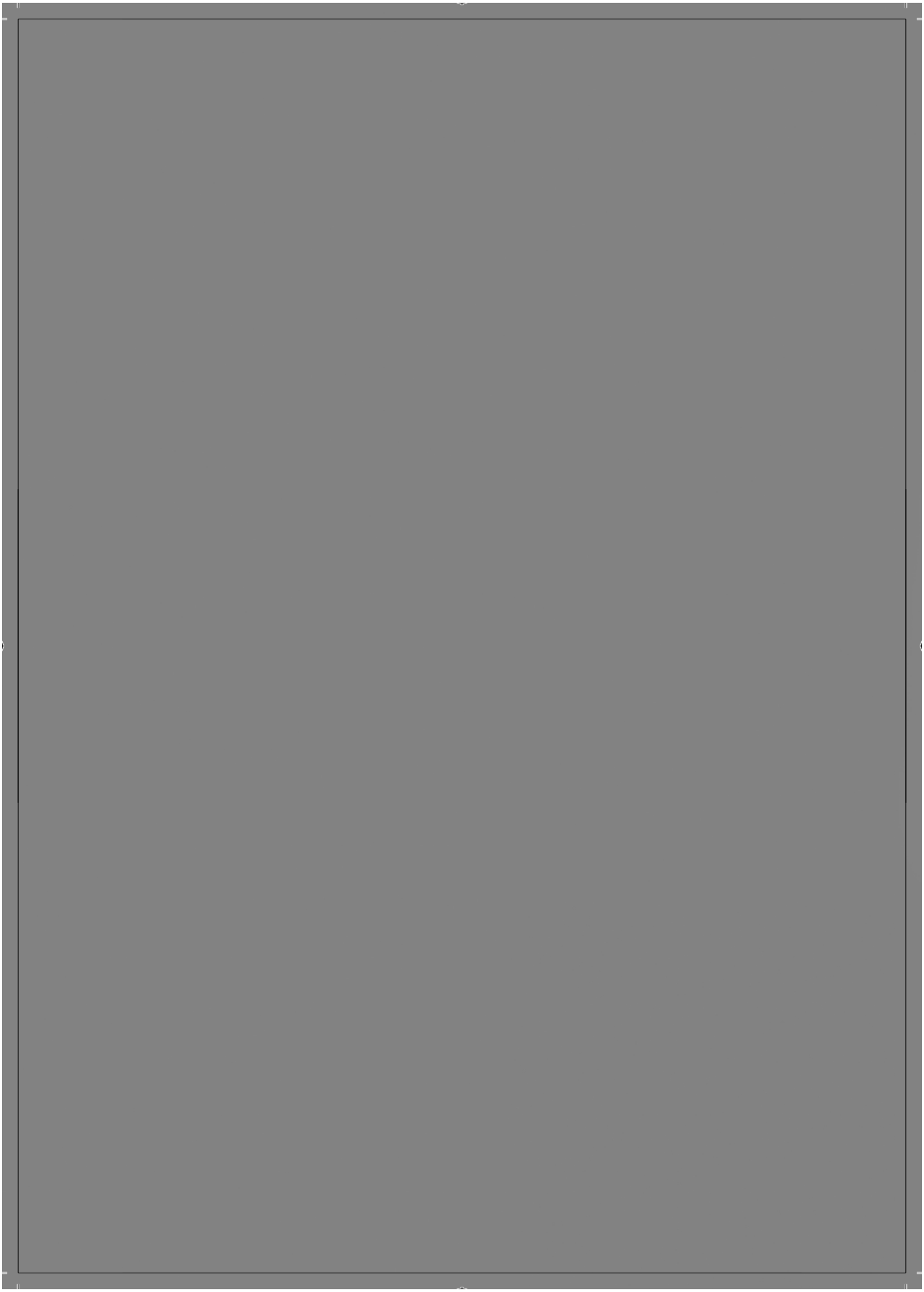

Chapter 5

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

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Abstract

Background: Statins show anticancer activity in melanoma cells. We investigated the association between statins and incidence and Breslow thickness of cutaneous melanoma (CM).

Patients and 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 ≥ 18 years and had ≥ 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 estrogens.

Results: Finally, 1318 cases and 6786 controls were selected. CM risk was not associated with statin use (≥ 0.5 year) (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, $p = 0.028$).

Conclusion: Our study suggests protective effects of statins on melanoma progression.

Introduction

Cutaneous melanoma (CM) accounts for 77 percent of all deaths due to skin cancer. The incidence of CM is increasing considerably, about 3 percent 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 posttranslational activation of several intracellular proteins 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 ratio (OR) = 0.63; 95% confidence interval (CI) = 0.22-1.84). [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.

Patients and methods

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 computerized 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 Netherlands Cancer Registry.

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 last date in PHARMO or date of metastasis, which ever 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 interobserver 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 three 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 three 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).

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

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 estrogens (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 estrogens 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 categorized 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.

Statistical analysis

Because CM may behave differently across gender, we analyzed the total study population, but also men and women separately. To test for statistical differences, χ^2 and Student's *t-test*s 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 categorized 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).

Results

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 three years before CM diagnosis. Accordance between the two authors in a random sample of 300 cases was high (Kappa values > 0.85), suggesting small interobserver variation. Of the 16133 controls matched on gender, age (± 2 years) and geographical region, 6786 (42.1%) could be included in the study (Fig. 1).

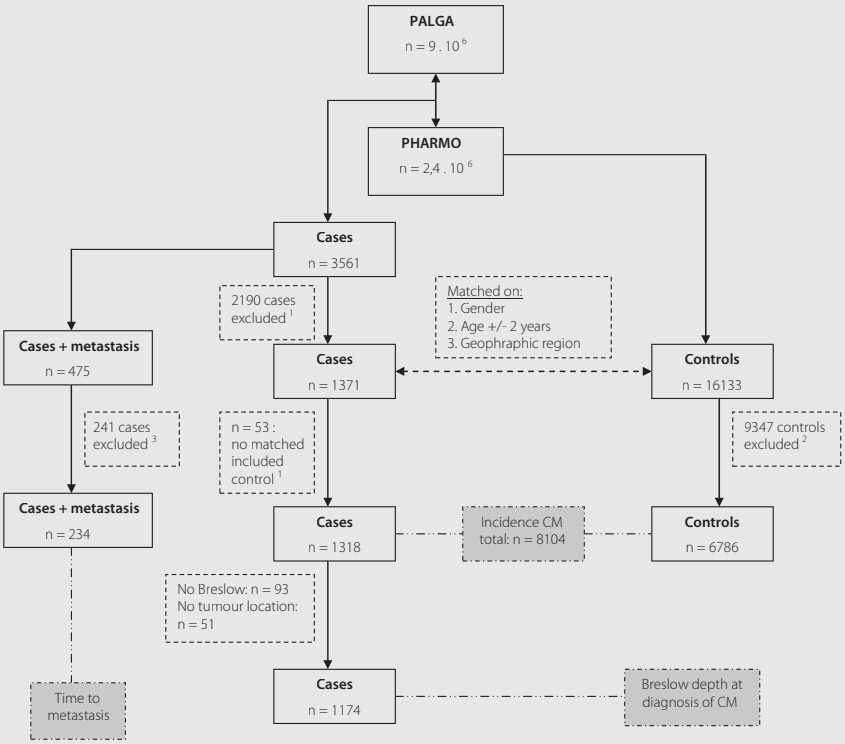
Risk of CM development and statin use

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 1.38 DDD/day [standard deviation (SD) 0.82 DDD/day]. Comparing prior drug use demonstrated significantly more use of NSAIDs and estrogens in the 3 years prior to diagnosis among CM patients (Tables 1A and Table 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 Table 1A and Table 1B remained significant after adjusting for confounding variables. Compared to female non statin users, women who had 3 or 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.

Figure 1 Flow chart study population



[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 %**

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 (± 1.52)	0.61 (± 1.55)	*0.04*
NSAIDs [a]	Yes	627 (47.6%)	2942 (43.4%)	
	No	691 (52.4%)	3844 (56.6%)	*0.01*
Estrogens [a]	Yes	264 (20.0%)	1117 (16.5%)	
	No	1054 (80.0%)	5669 (83.5%)	*0.002*
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%)	*0.21*
	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*

Table 1A Continued

		Cases n = 1318	Controls n = 6786	*p-value*
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 three 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.

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 was located on the trunk or extremities. Tumor characteristics such as Breslow depth, CM subtype and body location differed significantly between males and females. Tumor 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 4 dispenses or 0.66 DDD in average day dose was associated with a significantly reduced Breslow thickness (–10.7%, 95% CI = –18.5%, –2.2%, $p = 0.015$ and –11.0%, 95% CI = –19.7%, –1.2%, $p = 0.03$, respectively).

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*
Estrogens[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*

Table 1B Continued

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 amount dispensed 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	1.00	referent
	>0.5 yr	0.98	0.78 – 1.2
No. of Dispenses	0	1.0	referent
	1–8	1.0	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	1.0	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	1.0	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 estrogens.
[b] Time interval between estimated last day of use (based on last dispense and amount dispensed) and date of diagnosis of CM.

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	0.8	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 estrogens.
[c] Time interval between estimated last day of use (based on last dispense and amount dispensed) and date of diagnosis of CM.

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).

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	<0.001 [a]
Breslow in AJCC Categories				
0–1 mm	604 (51.4%)	223 (45.8%)	381 (55.5%)	
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%)	0.001 [b]
Type of melanoma				
Superficial spreading	784 (66.8%)	315 (64.7%)	469 (68.3%)	
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%)	0.02 [b]
Regression of primary tumor				
Yes	80 (6.8%)	31 (6.4%)	49 (7.1%)	
No / Unknown	1094 (93.2%)	456 (93.6%)	638 (92.9%)	0.61 [b]
Location of primary tumor				
Head / neck	160 (13.6%)	86 (17.7%)	74 (10.8%)	
Trunk	490 (41.7%)	270 (55.4%)	220 (32.0%)	
Extremity	524 (44.6%)	131 (26.9%)	393 (57.2%)	<0.001 [b]

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

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

Figure 2

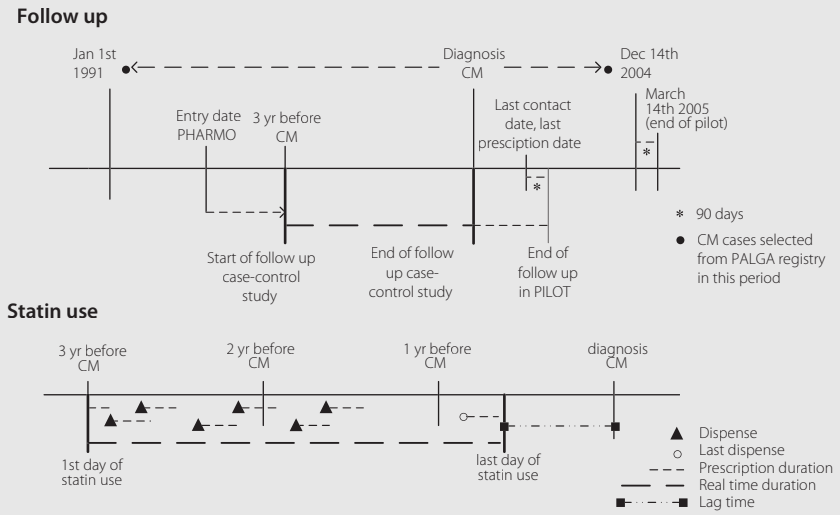
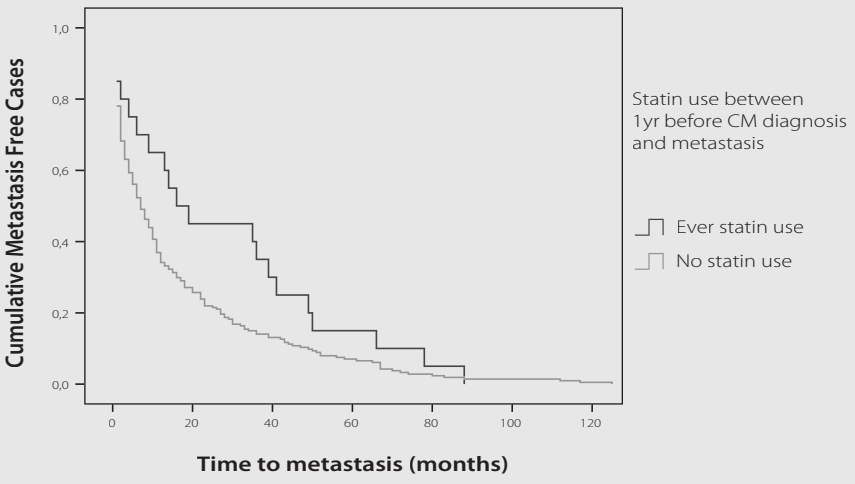


Figure 3



After adjustment for gender, age, year of CM diagnosis, body site, Breslow thickness, histological subtype, presence of regression, use of NSAID and estrogens 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).

Discussion

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 (Table 2A and Table 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]

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 year or more would result in a mean reduction of 0.58 mm. Because especially male cases 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 men is simply associated with earlier diagnosis of a CM lesion and not with slower progression of the CM lesion.

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.

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 generalized 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 one 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 to 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 randomized trial.

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.

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 randomized 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.

Acknowledgement

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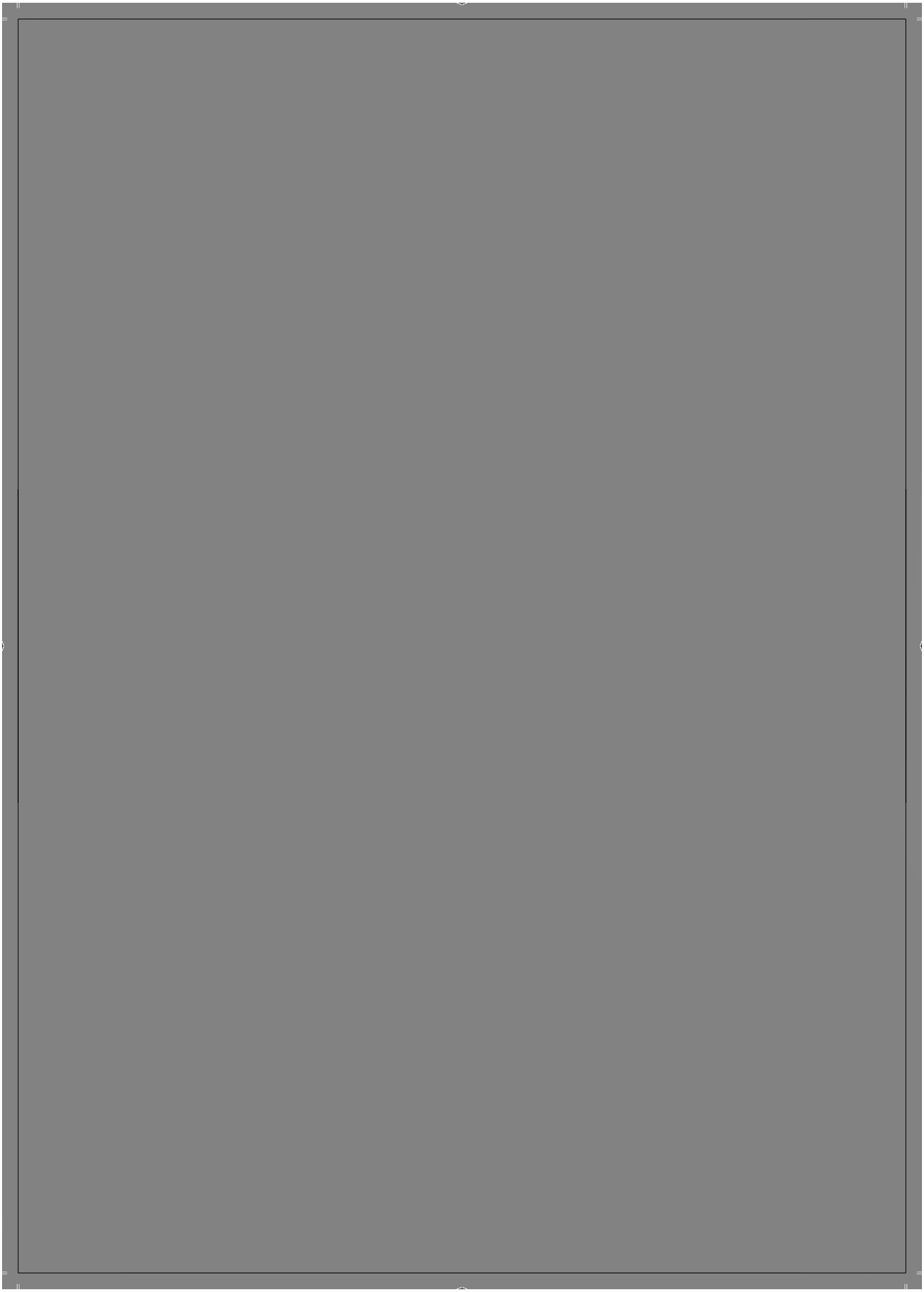

Chapter 6

Non-Steroidal Anti-Inflammatory Drugs and Melanoma Risk: Large Dutch Population-Based Case–Control Study

NSAIDs and melanoma risk

6

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Abstract

Background: This case-control study investigates the potential chemoprophylactic properties of non-steroidal anti-inflammatory drugs (NSAIDs) on the incidence of cutaneous melanoma (CM).

Patients and Methods: Data were extracted from the Dutch PHARMO pharmacy database and the PALGA pathology database. Cases had a primary CM between 1991 and 2004, were ≥ 18 years and were observed for 3 years in PHARMO before diagnosis. Controls were matched for date of birth, gender and geographical region. NSAIDs and acetylsalicylic acids (ASAs) were analyzed separately. Adjusted odds ratio (OR) and 95% confidence interval (CI) were calculated using multivariable logistic regression, and results were stratified across gender.

Results: 1318 CM cases and 6786 controls were eligible to enter the study. CM incidence was not significantly associated with ever ASA use (adjusted OR = 0.92, 95% CI = 0.76-1.12) or ever non-ASA NSAID use (adjusted OR = 1.10, 95% CI = 0.97-1.24). However, continuous use of low-dose ASAs was associated with a significant reduction of CM risk in women (adjusted OR = 0.54, 95% CI = 0.30-0.99) but not in men (adjusted OR = 1.01, 95% CI = 0.69-1.47). A significant trend (p = 0.04) from no use, non-continuous use to continuous use was observed in women.

Conclusion: Continuous use of low-dose ASAs may be associated with a reduced incidence of CM in women, but not in men.

Introduction

Cutaneous melanoma (CM) is a growing health problem, as CM incidence rates are steadily rising in both Europe [1] and the United States [2]. However, mortality rates seem to have leveled off, probably caused by increased awareness resulting in early detection of CM. [3] Although local CM is generally successfully treated with surgery, for metastatic disease therapeutic results remain disappointing. [1,4] Consequently, focus of melanoma research has shifted from therapy to prevention and early detection.

Chemoprevention may complement current preventive measures and is defined as the use of natural or synthetic agents to prevent, reverse, suppress or delay premalignant lesions from progressing into invasive cancer. [5] Non-steroidal anti-inflammatory drugs (NSAIDs) have shown promising results in various solid cancers [6] and may have chemopreventive potential in CM. [7] *In vitro* studies in melanoma cell lines have shown that NSAIDs can induce apoptosis [8,9] and inhibit tumor growth and invasion. [8,10,11]

The proposed anti-cancer mechanism of NSAIDs is inhibition of cyclooxygenase-2 (COX-2). This enzyme is inducable by inflammatory stimuli, is overexpressed in different neoplasms, and is probably linked to carcinogenesis through various mechanisms, for example, angiogenesis, apoptosis, inflammation, and immune function. [6, 12] However, NSAIDs may inhibit cancer through various COX-independent pathways as well. [13,14] This could be of particular importance in CM, as NSAIDs inhibit growth of CM cell lines independent of COX-2 [8-10,12,15] and COX-2 is not consistently expressed in CM. [9,11,16-18]

Thus far, most of the epidemiological studies assessing the chemoprophylactic effects of NSAIDs on CM incidence focus on acetylsalicylic acids (ASAs). A randomized controlled trial (RCT) and a large cohort study did not find an association between low- or high-dose aspirin use and CM incidence. [19,20] Studies investigating a possible association of CM and non-ASA NSAIDs are limited. Recently, a large cohort study did not observe an association with either ASA or non-ASA NSAIDs on CM incidence. [21] However, two smaller epidemiological studies suggested a reduced risk on CM incidence and progression in NSAID users. [22,23] Therefore, the potential chemoprophylactic properties of NSAIDs remain unclear due to heterogenity in study design and conflicting results.

The objective of this study is to investigate a possible protective effect of ASA and non-ASA NSAIDs on CM incidence in a large population-based sample by linking the Dutch pathology registry with a pharmacy database.

Patients and methods

Setting

This study was designed as a case-control study, using population-based data from two Dutch databases. PHARMO is a network of linked databases including a pharmacy database containing more than 2 million Dutch residents, representing 12% of the total Dutch population. The residents were included regardless of type of insurance. [24] An individual enters the PHARMO database when obtaining the first prescription in a PHARMO pharmacy, and is observed until the last prescription. As most patients in The Netherlands visit a single pharmacy, drug-dispensing records are virtually complete. [25] The prospectively gathered computerized drug-dispensing records contain the date of dispense, type, quantity, dosage form, strength, and daily dose of the prescribed drug.

PHARMO was linked to PALGA, the Dutch registry of histo- and cytopathology, using a variation of a reliable probabilistic algorithm. PALGA contains abstracts of all Dutch pathology reports encrypted with patient identification and diagnostic terms in scope with the SNOMED classification, and reached 100% participation from 1990 onwards, and therefore is the basis of the Netherlands Cancer Registry. [26]

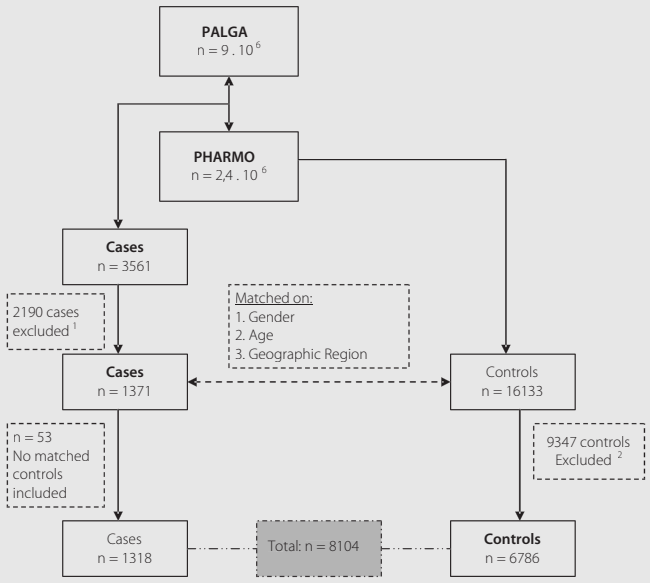
The protocol of this study was approved by the scientific and privacy committees of both PALGA and PHARMO, and was granted exempt status by the ethics board of the Leiden University Medical Centre.

Study population

Cases were defined as individuals with a CM diagnosis in PALGA between January 1st 1991 and December 14th 2004 and who were also registered in PHARMO in this period. The endpoint of the observation period up was defined as the date of CM diagnosis (index date). For each case, two investigators (AJ, ERK) extracted final diagnosis, date and Breslow's depth from the PALGA pathology reports with high accordance (kappa values > 0.85). [27] Cases were excluded if, in PALGA, the date of primary CM diagnosis was before the age of 18 years or before January 1st 1991, the primary melanoma was not pathologically confirmed, was *in situ*, or was non-cutaneous, or in PHARMO, the date of entry was unknown, gender was unknown, or time of observation before CM diagnosis was < 3 years (Fig. 1) .

For every eligible case, an average of five controls matched for gender, date of birth (± 2 years) and geographic region (~100 regions based on clusters of local pharmacies) was sampled from PHARMO. To calculate the time of observation for the controls, they were assigned the index date of the matched case to be able to determine the

Figure 1



1 Cases Excluded	Total	n=2243	100 %
1 = Entry date missing		n = 78	3.5 %
2 = Age at diagnosis <18 yrs		n = 19	0.8 %
3 = OP 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 (e.g. eye)		n = 205	9.1%
7 = in situ CM		n = 44	2.0%
8 = No matched controls included		n = 53	2.4 %

2 Controls Excluded	Total	n=9347	100 %
1 = Entry date missing		n = 640	6.8 %
2 = Age at diagnosis < 18 yrs		n = 30	0.3 %
3 = OP 3 yr before index date incomplete		n = 8673	93 %
4 = possible CM patient (ICD code 'CM')		n = 4	0.04 %

3 year observation period. Controls were excluded if, in PHARMO, the date of entry was unknown, they were younger than 18 years at the index date, the time of observation before index date was < 3 years, or a diagnosis of melanoma was recorded according to the International Classification of Disease (ICD9-CM) in the hospital linkage database of PHARMO (Fig. 1).

Drug Exposure

For all cases and controls, systemic NSAID use, restricted to the 3-year observation period before the index date, was extracted from the PHARMO database using the anatomical therapeutical classification (ATC) codes of the World Health Organization (WHO). All NSAIDs, including ASAs, available in The Netherlands were included (Table 1). Drug dispenses containing < 7 pills were excluded (for example, after a dental extraction), but weekly prescribed NSAIDs were included (for example, weekly pharmacy deliveries to nursery homes).

ASAs were investigated separately from non-ASA NSAIDs because, next to COX-2 inhibition, they inhibit thrombocyte aggregation, which has been linked to carcinogenesis. [28] Furthermore, ASAs are almost exclusively prescribed for long-term continuous use and not for intermittent use as an analgetic, in contrast with non-ASA NSAIDs.

ASA Use

Among all users, ASA use was categorized by prescribed dosage. Individuals who used low-dose ASA (30-100 mg daily) were categorized in continuous (that is, use of ≥ 990 U of ASA during the observation period of 3 years or 1095 days) and non-continuous users. Higher doses of ASA (≥100 mg) were dispensed far less frequently and were mostly prescribed for on-demand use, suggesting temporary use as an analgetic. It was not possible to extract continuous users from this group of high dose ASA users because of the low cumulative quantities of pills used during the observation period. Therefore, all users of high dose ASA were analyzed separately.

Non-ASA NSAID use

Non-ASA NSAIDs, such as ibuprofen and diclofenac, were prescribed irregularly, with a wide variation of daily prescribed doses, and to be used on demand. Therefore, assumptions for continuous or non-continuous use could not be made, and categorization was limited to the number of pills prescribed. For the categories of cumulative number of pills, the cutoff values were chosen to reflect levels of exposure: non-users, individuals who were likely to be exposed for < 2/3 of the observation period of 3 years (1-600 pills during 1095 days), individuals using on average more than one pill daily in 3 years (>1000 pills) and an intermediate group.

Potential confounders

Ever use of drugs related to progression and development of CM, such as statins [27] and estrogens [29], were considered possible confounders. The use of heparins,

Table 1 ATC codes and corresponding NSAID

ASAs	**ATC code**	**% of total** [1]
Acetylsalicylic acid	B01AC06 / N02BA01	22,5
Carbasalate calcium	B01AC08 / N02BA15	19,1
Total ASA use		***41.6***
Non-ASA NSAIDs	**ATC code**	**% of total** [2]
Diclofenac	M01AB05	20,5
Ibuprofen	M01AE01	14,5
Naproxen	M01AE02	10,0
Rofecoxib [3]	M01AH02	3,0
Diclofenac, combinations	M01AB55	2,5
Indometacin	M01AB01	2,3
Meloxicam	M01AC06	1,6
Piroxicam	M01AC01	1,2
Nabumetone	M01AX01	1,0
Ketoprofen	M01AE03	0,4
Celecoxib	M01AH01	0,3
Sulindac	M01AB02	0,3
Tiaprofenic acid	M01AE11	0,2
Aceclofenac	M01AB16	0,1
Etoricoxib	M01AH05	0,1
Flurbiprofen	M01AE09	0,1
Tenoxicam	M01AC02	0,1
Dexibuprofen	M01AE14	<0,1
Dexketoprofen	M01AE17	<0,1
Diflunisal	N02BA11	<0,1
Tolfenamic acid	M01AG02	<0,1
Metamizole sodium	N02BB02	<0,1
Total Non ASA NSAID use		***58.4***

[1] All available NSAID ATC codes were included in the study. Presented are ATC codes corresponding with 1 or more prescription among cases and controls.
[2] Percentage of the total 22,279 prescriptions among cases and controls.
[3] Withdrawn from the Dutch market in 2004.

ASAs = Acetylsalicylic acids; ATC = anatomical therapeutic chemical classification system; NSAIDs = non-steroidal anti-inflammatory drugs.

Table 2 Study Group Characteristics

		Total Group			Males			Females		
		Cases n = 1318	Controls n = 6786	p-value	Cases n = 540	Controls n = 2714	p-value	Cases n = 778	Controls n = 4072	p-value
Gender [1]	male	540 (41.0%)	2714 (40.0%)							
	female	778 (59.0%)	4072 (60.0%)	0.51						
Age at index date [2]	yrs	55.3 (± 15.9)	55.9 (± 15.5)	0.18	57.7 (± 14.6)	58.0 (± 14.2)	0.72	53.6 (± 16.5)	54.6 (± 16.1)	0.13
Total unique diagnoses [2]	no.	0.71 (± 1.52)	0.61 (± 1.55)	0.04	0.84 (± 1.8)	0.66 (± 1.6)	0.02 [3]	0.62 (± 1.3)	0.59 (± 1.5)	0.55 [3]
Total unique medications [2]	no.	7.53 (± 6.49)	6.93 (± 6.78)	<0.01	6.95 (± 6.9)	6.24 (± 6.3)	0.03 [3]	7.93 (± 6.2)	7.39 (± 7.0)	0.03 [3]
Estrogen use [1]	Ever Use	246 (18.7%)	1009 (14.9%)		-	-		246 (31.6%)	1009 (24.8%)	
	Never Use	1072 (81.3%)	5777 (85.1%)	<0.01	-	-		532 (68.4%)	3063 (75.2%)	<0.01
Statin use [1]	Ever Use	115 (8.7%)	574 (8.5%)		75 (13.9%)	309 (11.4%)		40 (5.1%)	265 (6.5%)	
	Never Use	1203 (91.3%)	6212 (91.5%)	0.75	465 (86.1%)	2405 (88.6%)	0.10	738 (94.9%)	3807 (93.5%)	0.15
ASA use	Never Use	1137 (86.3%)	5853 (86.3%)		435 (80.6%)	2219 (81.8%)		702 (90.2%)	3634 (89.2%)	
	Ever Use	181 (13.7%)	933 (13.7%)	0.99	105 (19.4%)	495 (18.2%)	0.51	76 (9.8%)	438 (10.8%)	0.41
Type of ASA use	Never use	1137 (86.3%)	5853 (86.3%)		435 (80.6%)	2219 (81.8%)		702 (90.2%)	3634 (89.2%)	
	Low-dose non-continuous	76 (5.8%)	455 (6.7%	0.24	42 (7.8%)	239 (8.8%)	0.53	34 (4.4%)	216 (5.3%)	0.28
	Low-dose continuous	61 (4.6%)	329 (4.8%)	0.75	48 (8.9%)	204 (7.5%)	0.28	13 (1.7%)	125 (3.1%)	0.03
	High dose	44 (3.3%)	149 (2.2%)	0.02	15 (2.8%)	52 (1.9%)	0.19	29 (3.7%)	97 (2.4%)	0.04
Non-ASA NSAIDs use	Never Use	700 (53.1%)	3862 (56.9%)		304 (56.3%)	1598 (58.9%)		396 (50.9%)	2264 (55.6%)	
	Ever Use	618 (46.9%)	2924 (43.1%)	0.01	236 (43.7%)	1116(41.1%)	0.27	382 (49.1%)	1808 (44.4%)	0.02

Cumulative nr. of pills	No use	700 (53.1%)	3862 (56.9%)		304 (56.3%)	1598 (58.9%)		396 (50.9%)	2264 (55.6%)		
	1-600	588 (44.6%)	2728 (40.2%)	<0.01	226 (41.9%)	1051 (38.7%)	0.20	362 (46.5%)	1677 (41.2%)	<0.01	
	601-1000	12 (0.9%0	92 (1.4%)	0.29	3 (0.6%)	31 (1.1%)	0.26	9 (1.2%)	61 (1.5%)	0.63	
	>1000	18 (1.4%)	104 (1.5%)	0.86	7 (1.4%)	34 (1.3%)	0.85	11 (1.4%)	70 (1.7%)	0.74	

[1] Number of cases and controls presented, ± SD tested for statistical difference with χ^2-test.
[2] Mean value presented, tested for statistical difference with t-test
[3] Equal variances not assumed according to Levene's test for Equality of variances

ASA = acetylsalicylic acid, NSAIDs = non-steroidal anti-inflammatory drugs.

fibrates, and other llipid-lowering drugs was also recorded. However, the number of individuals using these drugs was too small (<1.0%) to be used in further analysis. To adjust for a possible surveillance bias (that is, patients who seek medical care are more likely to be diagnosed with other disease including CM), a proxy of health-care and pharmacy-seeking behaviour was created by calculating the total number of unique ATC codes (excluding all NSAIDs) and the total number of unique ICD9-CM codes (that were primary discharge diagnosis after hospitalization) which were both recorded in the database in the 3 years before the index date. The ICD code for melanoma found for each case was not included in the total number of unique ICD codes to avoid overmatching. Both confounders proved to be significant in all multivariable analyses performed and also showed a significant interaction with each other. This interaction term was added in the multivariable analysis (p <0.01).

Statistical Analysis

A chi-square test was used to test for statistical differences between categorical variables, for continuous variables a Student's *t*-test or a Mann-Whitney U test was used as appropriate. A multivariable logistic regression model was used to calculate adjusted odds ratios (OR) and 95% confidence intervals (CI) to analyze the association between dependent CM incidence and NSAID use and its defined categorizations of exposure.

As CM development, progression and survival, as the effect of potential chemoprophylactic drugs, may differ across gender [27,28,30,31], a pre-specified separate analysis for men, women and the total group was carried out.

All statistical tests were two sided, with a rejection of the null hypothesis at p <0.05. All statistical analyses were performed using SPSS 14.0 (.2) (SPSS Inc. Chicago, IL).

Results

Study population

The ascertainment of the cases and controls has been described previously. [27] Briefly, of the 3561 subjects who were registered in PHARMO (Institute for Drug Outcome Research) and had a systemized nomenclature of medicine (SNOMED) code 'melanoma' in PALGA (the natoinwide network and registry of histo- and cytopathology in The Netherlands), 1318 cases (37.0%) met the eligibility criteria (Fig. 1). Patients were mostly excluded because the registration periods in PHARMO and PALGA did not match, leading to incomplete pharmacy records in PHARMO in the 3-year observation period before CM diagnosis. Of the 16133 controls matched on gender, age and geographical region, 6786 (42.1%) met the inclusion criteria.

About 60% of the study population was female, with a mean age of 55 years (Table 2). Compared with the controls, cases had a significantly higher number of unique non-melanoma international classification of disease (ICD) diagnoses (0.71 versus 0.61, $p = 0.04$), which was confirmed in men, but not in women. Also, cases had a higher number of unique medications prescribed (7.53 unique ATC codes versus 6.93, $p < 0.01$), which was confirmed in both men and women. As reported earlier, women with melanoma used more estrogens compared to the control population (31.6% versus 24.8%, $p < 0.001$). [29]

ASA use and CM incidence

More than 40% of the total NSAID use consisted of ASA use (Table 1). The proportion of CM patients who used ASA was comparable to the controls, except for high dose ASAs (Table 2). Female cases were significantly less likely to be a continuous user of low-dose ASAs than their matched controls (1.7% versus 3.1%, $p = 0.03$). In men, no significant difference in the distribution of ASA exposure was observed. After adjusting for age, gender, year of diagnosis, prior use of statins and estrogens, and unique number of ICD and ATC codes in a multivariable model, none of the ASA exposure variables was significantly associated with CM incidence in the total study population and in men (Table 3). However, in women, continuous use of low-dose ASA for 3 years was associated with a reduced risk of developing a CM of almost 50% (adjusted OR = 0.54, 95% CI = 0.30-0.99). In addition, in women, there was a significant dose-response trend for no use, non-continuous use, and continuous use (p-value for trend=0.04).

Table 3 ASA / NSAID use and cutaneous melanoma

		Total			Males			Females		
		n	*Adjusted OR*[1]	*95% CI*	*n*	*Adjusted OR*[1]	*95% CI*	*n*	*Adjusted OR*[1]	*95% CI*
ASA use	*Overall Exposure*									
	Never ASA use	6990	1.00	*referent*	2654	1.00	*referent*	4336	1.00	*referent*
	ASA use	1114	0.92	0.76-1.12	600	0.92	0.69-1.21	514	0.90	0.68-1.19
	Use of ASA									
	Never Use	6990	1.00	*referent*	2654	1.00	Referent	4336	1.00	*referent*
	Low dose < 3 yrs [1]	531	0.77	0.58-1.01	281	0.72	0.49-1.06	250	0.82	0.55-1.22
	Low Dose 3 yrs [1]	390	0.87	0.64-1.18	252	1.01	0.69-1.47	138	**0.54**	**0.30-0.99**
	High dose (ever) [2]	193	1.35	0.96-1.92	67	1.34	0.74-2.43	126	1.37	0.89-2.11
Non-ASA NSAIDs	*Overall Exposure*									
	Never NSAID use	4562	1.00	*referent*	1902	1.00	*referent*	2660	1.00	*referent*
	NSAID use	3542	1.10	0.97-1.24	1352	1.04	0.86-1.26	2190	1.13	0.96-1.34
	Cumulative Pills									
	0	4562	1.00	*referent*	1902	1.00	*referent*	2660	1.00	*referent*
	1-600	3316	1.12	0.98-1.23	1277	1.06	0.87-1.27	2039	1.15	0.98-1.36
	601-1000	104	0.67	0.36-1.23	34	0.46	0.14-1.51	70	0.82	0.40-1.69
	>1000	122	0.89	0.53-1.43	41	0.96	0.42-2.21	81	0.88	0.46-1.69

[1] Adjusted for age, sex (only in total group), year of diagnosis, the use of statins resp. estrogens (only in females), the total of different medical diagnoses, total of different medications prescribed and the interaction term between the latter two.
[2] use of 30-100 milligrams acetylsalicylic acid per unit (≥ 990 pills is considered 3 years –continuous- use).
[3] use of >100 milligrams acetylsalicylic acid per unit.

OR = odd ratio, CI = confidence interval, ASA = acetylsalicylic acid, NSAIDs = non-steroidal anti-inflammatory drugs.

Non-ASA NSAID use and CM incidence

The most commonly dispensed non-ASA NSAIDs were diclofenac (20,5%), ibuprofen (14,5%) and naproxen (10.0%) (Table 1). Female and, to a lesser extent, male CM patients were more likely to have ever used non-ASA NSAIDs compared to controls (Table 2). Of the non-ASA NSAID users, the overwhelming majority used < 600 pills during 3 years and only 2.3% and 2.9% of cases and controls, respectively, used more than 600 pills. In the distribution of the categories of the cumulative number of pills, the only significant difference was observed in the lowest category of 1-600 pills for the total study population and women.

In the multivariable models that adjusted for multiple confounders, no significant associations were found, although relative low non-ASA NSAID exposure (1-600 pills) was borderline significantly associated with a modest increase in CM risk (OR = 1.12, 95% CI = 0.98-1.23, Table 3). In further subgroup analysis, the use of 1-4 prescriptions of non-ASA NSAIDs in 3 years was significantly associated with a marginally increased risk of CM (OR = 1.15, 95% CI = 1.01-1.30, data not shown). Higher levels of exposure appeared to be protective for all subgroups, but none of these associations were significant (Table 3).

Discussion

NSAID use and risk of CM

Continuous use of low-dose ASAs during 3 years was associated with a reduced likelihood of developing CM in women but not in men.

In contrast, none of the non-ASA NSAID variables were significantly associated with risk of having a CM in the multivariable model (Table 3). However, infrequent use of pills (1-600 pills in 3 years), was significantly associated with the incidence of CM in univariate analysis (Table 2), but this was not significant in the multivariable model after adjusting for health-care consumption (Table 3), suggesting that this and possibly other confounders affected the univiarate model. Interestingly, a similar association has been reported in a case-control study in prostate cancer. [33] This illustrates that health care utilization may be an important confounder in pharmaco-epidemiological studies.

The use of larger quantities of non-ASA NSAIDs (>600 pills in 3 years) seemed to be protective for CM but did not reach significance, which could be explained, in part, by a relatively short time of observation (3 years), limited sample size in this subgroup (<225 patients), and/or that non-ASA NSAIDs were administered as analgetics (the

prescribed frequency of use by physicians was most often 'when needed'); thus implying non-continuous exposure. On account of small numbers, separate analyses for selective-COX-2 inhibitors could not be carried out.

The observed difference in chemoprophylactic effects between non-ASA NSAIDs and ASAs may be dependent on the patterns of use or on a different mechanism of action. First, low-dose ASAs are most commonly prescribed as daily cardiovascular preventive drugs, whereas non-ASA NSAIDs and high dose ASAs are commonly used irregularly as analgetics. Second, ASAs may have additional anti-cancer effects in comparison to non-ASAs, such as inhibition of thrombocyte-aggregation [28], or effects cancer-related systems as apoptosis, NF-κB, DNA-repair systems, oxidative stress, or mitochondrial calcium uptake. [14]

We did not find a reduced CM incidence among overall non-ASA NSAID or ASA users, which is in accordance with three large observational studies. A large cohort study of regular and high dose ASA (>325 mg) exposure observed no protective effect on CM. [20] A second cohort confirmed the absence of an association between ASA or non-ASA NSAID use and CM incidence. [21] This study, however, has several limitations, that is, low-dose aspirin exposure was excluded in subgroup analyses, ~40% of cases were CM *in situ*, and stratification across gender was not carried out. Our results, showing an association of low-dose ASA use in women with CM is in contrast with an RCT among females for whom low-dose aspirin use (100 mg every other day) for an average of 10 years did not affect CM incidence (68 versus 70 incident cases, $p = 0.87$). [19] This study however was limited by a small number of CM cases, non-continuous exposure, and was not population-based.

In other malignancies, multiple studies investigating the chemopreventive properties of ASA and non-ASA NSAIDs have been published. A review showed that in colorectal, breast and lung cancer, the risk reductions by non-ASA NSAIDs and ASAs were comparable [6], which contradicts our results that suggest a different effect. Results of a case-control study on prostate cancer, however, were comparable: prolonged use of ASAs showed a protective effect, whereas use of non-ASA NSAIDs did not. [33] In lung [34], breast [35] and prostate [33] cancer, exposure to regular or high-dose use of ASAs did, but exposure to low-dose ASA did not, decrease the incidence of these cancers, which is not in line with our findings in CM patients.

However, comparing the results of studies assessing the chemoprophylactic effect of NSAIDs is challenging because studies differ in several important ways such as ascertainment of drug exposure (for example, self-reported or pharmacy database), definition of exposure, type of NSAID (ASA or non-ASA), dose, duration, patterns of use (for example, sporadic, intermittent, chronic), drug adherence, study population (for example, general population, cohorts from tertiary centers), melanoma (for example, invasive or *in situ* CM), sample size, and subgroup analyses (that is, stratification across gender). A pivotal unresolved problem is the definition of the dosage of NSAID, which could have chemoprophylactic effects.

Gender differences

Stratification across gender showed a gender difference in favor of women, especially for continuous use of low-dose ASAs. This apparent discrepancy between men and women is not well understood and may be explained by pharmacological and melanoma differences. Pharmacodynamics and pharmacokinetics of ASA differ between men and women: the effect on platelets differs across gender and women achieve higher concentrations with equal doses being administrated. [32] As ASA may influence oxidative stress, the gender difference in antioxidant enzymes may have a role. [36] Remarkably, a recent RCT investigating antioxidant supplementation showed an increase of CM incidence in women, but not in men. [37] Another explanation may be that melanoma biology itself may not be comparable in men and women, as CM survival differs significantly across gender when adjusted for other prognostic factors. [30,31] Differences in adherence to cardiovascular drugs, however, are not likely to explain the observed gender differences. [38]

Interestingly, we previously reported a gender difference in the effects of statins on CM incidence and progression using the same study population. [27] Future (epidemiological) studies are warranted to explore CM gender differences.

Strengths and weaknesses

This is the largest population-based study that investigates the effect of NSAID use on CM incidence in more than 1350 cases. The CM cases were confirmed by a pathology report, and drug exposure was prospectively assessed by a highly reliable pharmacy database. [39] In PHARMO, detailed information on drug use was available, such as the number of dispenses, the number of dispensed pills, and dosage. As the dosages (in WHO's defined daily doses) of NSAIDs differ largely between the indications for which they are prescribed, we were not able to include this information. Furthermore, since a large proportion of the NSAIDs are used as analgetics 'on demand', no data were

available regarding the duration of use for these types of NSAIDs. Therefore, duration of use could not be included in the analyses, except based on the number of pills prescribed. As several NSAIDs are available over the counter without a prescription, the actual use of NSAIDs is underestimated. Therefore, if this would influence our results, it is most likely that this would produce bias toward the null. However, this misclassification is likely to be equal among cases and controls; hence, bias is likely to be minimal. In this study, NSAID use was ascertained in the 3 years before CM diagnosis, which may have been too short to detect the effect of NSAID exposure. [6] However, increasing the observation period to 5 years decreased the sample size substantially (from 1318 to 931 CM cases). Although a proxy for health care consumption was included in the multivariable model, surveillance bias may still have affected our results. Information on life-style factors such as sun exposure was not available, but the confounding effect of sun exposure on NSAID use seems to be limited.

Conclusion

In conclusion, long-term use of (low-dose) ASA was associated with a reduced risk of CM in women, but not in men. Future observational and ultimately interventional clinical studies are needed to confirm these findings.

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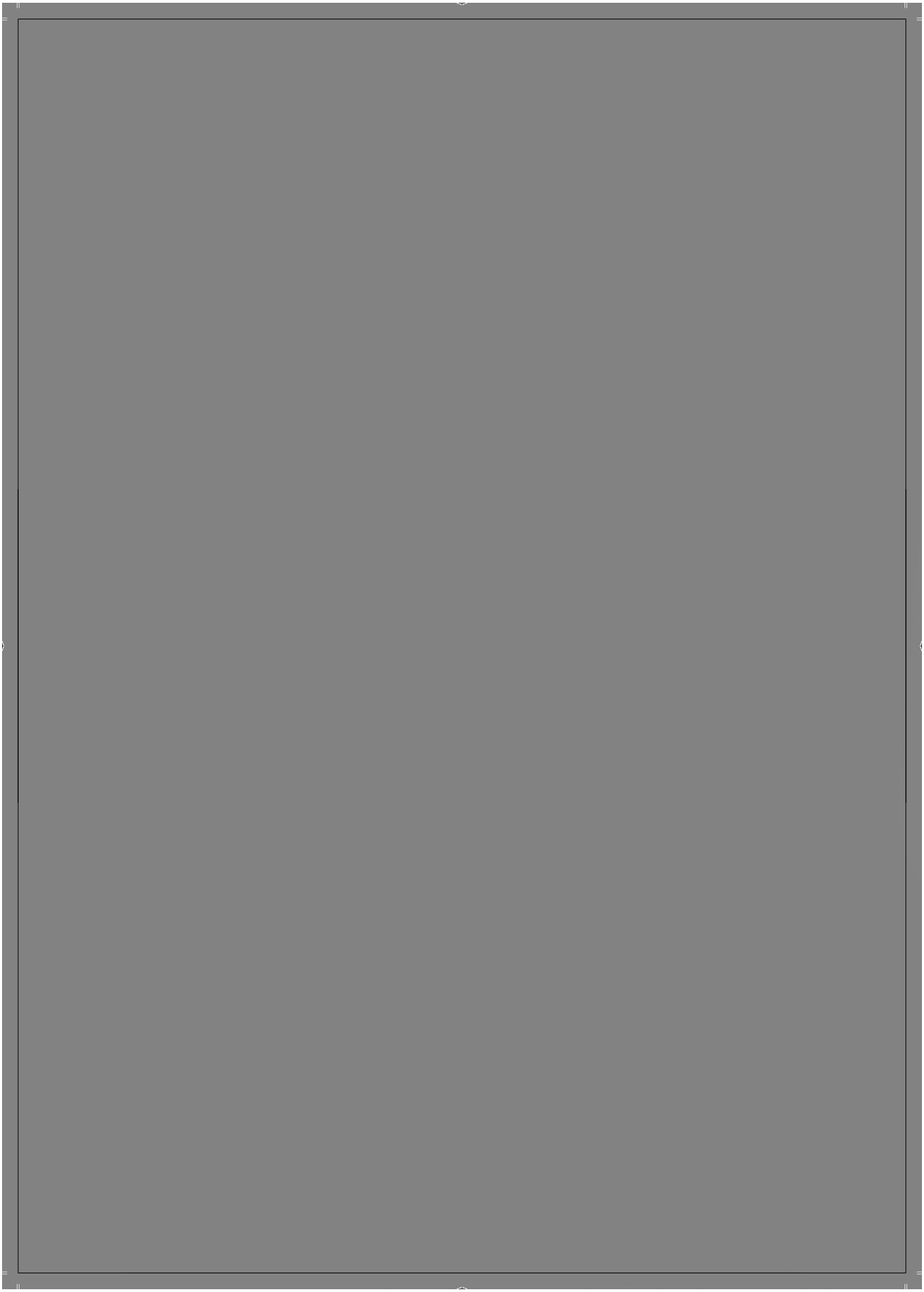

Chapter 7

Melanoma Incidence and Exposure to Angiotensin-converting enzyme Inhibitors and Angiotensin Receptor Blockers

7

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Abstract

Background: A reduced incidence of nonmelanoma skin cancer among users of Angiotensin-converting enzyme inhibitors (ACEi) and Angiotensin Receptor blockers (ARb) has been reported. A similar effect is suggested for cutaneous melanoma. We aimed to investigate the possible association between use of ACEi and ARb and the risk of cutaneous melanoma .

Patients and Methods: A general population-based case control study with the PHARMO database, containing drug-dispensing records from community pharmacies and the national pathology database (PALGA) was conducted. Cases were patients with a primary cutaneous melanoma between January 1st 1991 and December 14th 2004, aged ≥ 18 years and having ≥ 3 years of follow-up prior to diagnosis.

Results: Finally, 1272 cases and 6520 matched controls were included. Multivariable conditional logistic regression showed no statistically significant associations between the incidence of melanoma and the use of ACEi (adjusted odds ratio (OR) = 1.0, 95% CI = 0.8-1.3) or ARb (adjusted OR = 1.0, 95% CI = 0.7-1.5).

Conclusion: In this study, the use of ACEi or ARb does not seem to protect against the development of cutaneous melanoma. However, we cannot exclude an association between ACEi and ARb exposure and an increased or decreased incidence of cutaneous melanoma.

Introduction

Chemopreventive effects in cancer have been suggested for angiotensin-converting enzyme (ACEi) inhibitors and angiotensin receptor (ARb) blockers in both *in vitro* studies, animal studies and epidemiologic studies. [1-6] *In vitro* and *in vivo* effects have been demonstrated on cell proliferation, gene expression, migration and invasion and angiogenesis. [1] These effects may be mediated through angiotensin II or bradykinin. [1] However, other mechanisms, such as inhibition of metalloproteases [4], reduction of the activity of plasminogen activator inhibitor-I [7], generation of angiostatin from plasmin [8] or activity as a free-radical scavenger [9] if a free sulhydryl donor is present in the molecule, e.g., captopril and zofenopril, may also be involved. Depending on which mechanisms are involved, chemopreventive effects may be considered to be an overall class effect for both ACEi and ARb, may be present for only ACEi or may be restricted to exposure to ACEi with a certain chemical structure.
In human head and neck squamous skin cancer cells, Yasumatsu and Nakashima observed a significant inhibitory effect on tumor growth and blood vessel formation mediated by perindopril. [5] Specifically for cutaneous melanoma, an *in vitro* study showed that captopril has antitumor activity in a human melanoma xenograft model. [10]
For melanoma, chemoprevention is of special interest because of rapidly increasing incidence (http://www.cancer.org/, Cancer Facts and Figures 2008, accessed February 3rd 2009) and the lack of survival prolonging therapies for advanced disease. [11] Recently, two epidemiological studies among users of ACEi and ARb reported on reduced risks of nonmelanoma skin cancer. [6,12]
With respect to the expected safety profile, ACEi and ARb would be good candidates because they are widely used in clinical practice with few side effects. However, to our knowledge, no observational studies have been performed that specifically investigate the chemoprophylactic properties of ACEi and ARb in melanoma. Therefore, we investigate the potential association between the risk of cutaneous melanoma and exposure to (different chemical drug classes of) angiotensin-converting enzyme inhibitors and angiotensin receptor blockers.

Patients and methods

Study design

We conducted a general population-based case control study exploring the use of ACEi and ARb among individuals with and without cutaneous melanoma. The protocol

of this study was approved by the scientific and privacy committees of both PALGA and PHARMO, and was granted exempt status by the ethics board of the Leiden University Medical Centre. An outline of the methods is presented here. Additional details are presented in earlier work. [13]

Data were extracted from the PHARMO (PHARmaco MOrbidity) linkage network and the PALGA database. PHARMO contains virtually complete drug-dispensing records of over 2 million Dutch residents, included regardless of the type of health insurance or other relevant factors and representing >12% of the Dutch population (http://www.pharmo.nl, Databases, accessed October 7th 2009). These computerized drug-dispensing histories contain all dispensed prescriptions and include type, quantity, dosage form, strength, dispensing date and prescribed daily dose of the dispensed drug.

PALGA is the Nationwide Network and Registry of Histo- and Cytopathology and contains pathology abstracts with diagnostic terms in scope with SNOMED classification of all Dutch patients (100% registration since 1990) and is the source of the Netherlands Cancer Registry. PHARMO and PALGA are linked using a variation of a reliable probabilistic algorithm. [14,15]

Two investigators read all pathology reports to validate the melanoma diagnoses. Interobserver variation was assessed on 300 randomly selected cases.

Cases were included if they had a primary diagnosis of cutaneous melanoma between January 1st 1991 and December 14th 2004 in PALGA, were aged 18 years or older at diagnosis and had at least 3 years of complete follow-up in PHARMO prior to diagnosis. For every case, an average of five controls, matched for age (± 2 years), gender, and geographical region, was included. To calculate follow-up, controls were assigned the index date of the matched case. Potential controls were excluded if, in PHARMO, a date of entry was unknown, they were younger than 18 years at the index date, 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. If more controls were elligible, the excess number of controls was randomly deleted.

Drug exposure

For cases and controls, dispenses of all commercially available ACEi and ARb (Anatomical Therapeutic Classification (ATC) codes: C09AAxx and C09CAxx), restricted to the 3-year observation period before the index date, were included. To avoid misclassifying cases and controls as ACEi or ARb users, drug exposure was defined as at least 6 months of cumulative prescription duration in the 3 years before melanoma

(e.g., after one or two first dispenses patients may discontinue for several reasons, effects on melanoma incidence of such short periods of use are considered to be unlikely).

ACEi were further classified in three drug classes according to their chemical structure (Table 1).

The level of exposure to ACEi and ARb was expressed in defined daily doses (DDD) according to WHO definitions (http://www.whocc.no/atcddd/indexdatabase/, last accessed February 6th 2009). Drug exposure was further detailed with three additional drug exposure variables, all with the 6-month threshold. In explanation, the cumulative dispensed dose, the cumulative prescribed duration and the average day dose within the 3-year period were calculated. The average day dose was defined as the cumulative dose divided by the cumulative duration. We categorized these drug exposure variables across tertiles or the median depending on the number of users.

Table 1 Chemical drug class and ATC codes for commercially available ACE inhibitors

ATC code	Generic drug name	Chemical drug class
C09AA01	Captopril	Sulfhydryl
C09AA02	Enalapril	Carboxyl
C09AA03	Lisinipril	Carboxyl
C09AA04	Perindopril	Carboxyl
C09AA05	Ramipril	Carboxyl
C09AA06	Quinapril	Carboxyl
C09AA07	Benazepril	Carboxyl
C09AA08	Cilazapril	Carboxyl
C09AA09	Fosinorpil	Phosphoryl
C09AA10	Trandalopril	Carboxyl
C09AA11	Spirapril	Carboxyl
C09AA13	Moexipril	Carboxyl
C09AA15	Zofenopril	Sulfhydryl

ATC code= Anatomical Therapeutic Classification code.

Potential confounders

Ever drug use of drugs possibly related to progression or development of melanoma, statins, estrogens and non-steroidal anti-inflammatory drugs were considered as potential confounders. [16] Use of fibrates and lipid-lowering drugs other than fibrates or statins was recorded, but the number of cases and controls using these drugs were too small to be used in further analysis. To test as an additional potential confounder, the total number of unique (singular) codes of the International Classification of Disease 9th revision, as an estimate of health care consumption which may affect the likelihood of melanoma diagnosis, was calculated for each participant in the 3 years before diagnosis.

Statistical analysis

A multivariable conditional logistic regression model was used to calculate adjusted OR and 95% confidence interval (CI) for the association between incident cutaneous melanoma and the use of ACEi and ARb. Potential confounders (the total number of unique medical diagnoses, ever drug use of in the 3-year period of respectively statins, estrogens, and non-steroidal anti-inflammatory drugs) were included in the multivariable model if they influenced the regression coefficient by 10% or more. [17] In sensitivity analyses, stratification across the chemical drug class was performed because some of the reported mechanisms of actions would predict chemopreventive effects only for ACEi with a certain chemical structure (see introduction). Additionally, separate analyses were performed for men and women. All statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL).

Results

Study population

In the baseline study, 1,318 melanoma cases and 6,786 matched controls were included.13 Of these, 46 cases and 266 controls were excluded because they used an ACEi or ARb for less than 6 months. The mean age of the cases and controls was respectively 54.9 years [standard deviation (SD) 15.9] and 55.5 years [SD 15.4] (Table 2). Accordance between two investigators on the validation of melanoma diagnosis was high (Kappa value > 0.85) in a random sample of 300 cases, suggesting small interobserver variation.

Exposure to ACE inhibitors and AR blockers

ACEi were used by 85 cases (7%) and 433 controls (7%). Among the ACEi users, 401 (92%) used a carboxyl derivative, 106 (24%) used a sulfhydryl derivative and 24 (6%)

Table 2 Characteristics of the study population

	Cases (n = 1272)		Controls (n = 6520)	
	n	**%**	**n**	**%**
Gender				
male	519	(41 %)	2598	(40 %)
female	753	(59 %)	3922	(60 %)
Drug use				
ACE inhibitor users [a]	85	(7 %)	433	(7 %)
Carboxyl [a,b]	65	(5 %)	332	(5 %)
Sulfhydryl [a,b]	19	(2 %)	87	(1 %)
Phosphoryl [a,b]	2	(0.2 %)	22	(0.3 %)
AR blocker [a]	30	(2 %)	148	(2 %)
estrogen users [c,d]	259	(34 %)	1090	(28 %)
statin users [c]	104	(8 %)	511	(8 %)
NSAID users [c]	591	(47 %)	2740	(42 %)
Total unique diagnoses				
mean number		0.7		0.6
Age at diagnosis [e]				
18-34 yr	134	(11 %)	579	(9 %)
35-44 yr	223	(18 %)	1125	(17 %)
45-54 yr	274	(22 %)	1445	(22 %)
55-64 yr	259	(20 %)	1384	(21 %)
65-74 yr	223	(18 %)	1159	(18 %)
75 yr and older	159	(13 %)	828	(13 %)

[a] At least 6 months of drug use.
[b] See Table 1 for chemical drug class classification of ACE inhibitors.
[c] Ever drug use.
[d] Females only, 753 cases and 3922 controls.
[e] Cases: mean ± standard deviation: 54.9 years ± 15.9 years and range: 18-94 years;
Controls: mean ± standard deviation: 55.5 years ± 15.4 years and range: 18-95 years.

ACE = Angiotensin-Concerting Enzyme, AR = Angiotensin Receptor,
NSAID = Non-steroidal Anti Inflammatory Drug.

used a phosphoryl derivative. ARb were used by 30 cases (2.5%) and 148 controls (2.4%). Cases and controls using ACEi or ARb was prescribed a median average day dose of 1.0 DDD per day (interquartile range: 0.7-2.0 DDD) and 1.0 DDD per day (interquartile range: 1.0-1.8 DDD), respectively. The use of ACEi was not significantly

associated with the incidence of cutaneous melanoma (adjusted OR = 1.0; 95% CI = 0.8-1.3). Increasing cumulative prescription duration, cumulative dose or average day dose also did not show a statistically significant effect of ACEi on cutaneous melanoma incidence (Table 3). After adjustment for age at melanoma diagnosis and the number of medical diagnoses, the use of ACEi was not associated with a decreased Breslow thickness (estimated percentage change in Breslow depth: 2.1%, 95% CI: -17.4% to 26.2%). We previously described the calculation method used. [13]

For ARb no significant associations were demonstrated if users (>0.5 year) are compared to non-users (adjusted OR = 1.1; 95% CI = 0.7-1.5) and if among them the cumulative prescription duration, cumulative dose or average day dose was compared (Table 3). The use of ARb was also not associated with a decreased Breslow thickness after adjustment for the number of medical diagnoses and age at melanoma diagnosis (2.4%, 95% CI = -25.3% to 40.6%).

Sensitivity analysis

Stratification across the three chemical drug classes was performed in a sensitivity analysis. For carboxyl and sulfhydryl ACEi, the association was similar to the results for the overall effect of ACEi (adjusted OR = 1.0, 95% CI = 0.8-1.3 and adjusted OR = 1.1, 95% CI =: 0.7-1.7, respectively). Very few cases and controls used phosphoryl ACEi, resulting in a large confidence interval (adjusted OR = 0.6, 95% CI = 0.1-2.4). Additionally, separate analyses for men and women, showed similar results (data not shown).

Discussion

In this study the use of ACEi or ARb does not seem to protect against the development of cutaneous melanoma. However, our study cannot exclude an association between ACEi and ARb exposure and either a (moderately) increased or decreased incidence of cutaneous melanoma. It is, for instance, possible that exposures with a longer duration or to higher doses of ACEi or ARb are needed for an association to be detected.

A decreased Breslow thickness among the cases using ACEi or ARb, as compared to the melanoma patients who did not use ACEi nor ARb, could be considered a clue for this possibility. However, the use of ACEi or ARb was not associated with decreased Breslow depth.

The major strengths of this study were the large population-based sample of pathology confirmed melanoma cases and the prospectively collected and detailed information about drug dispenses. A limitation of the study is the relatively small

Table 3 Prior Use of ACE Inhibitors or Angiotensin II Receptor Antagonists in the Study Population

	Cases		Controls		OR [a]	95% CI
USE OF ACE INHIBITORS [b]						
Users versus non-users	n=1272		n=6520			
	n	**%**	**n**	**%**		
non-exposed	1187	93.3 %	6087	93.4 %	1.0	referent
exposure >0.5 yr	85	6.7 %	433	6.6 %	1.0	0.8 – 1.3
Cumulative prescription duration [c]						
non-exposed	1187	93.3 %	6087	93.4 %	1.0	referent
non-exposed	32	2.5 %	151	2.3 %	1.1	0.7 – 1.5
1-750 days	19	1.5 %	100	1.5 %	1.0	0.6 – 1.5
751-1000 days	34	2.7 %	182	2.8 %	1.0	0.7 – 1.4
Cumulative dose						
0 DDD	1187	93.3 %	6087	93.4 %	1.0	referent
1-600 DDD	26	2.0 %	153	2.3 %	0.9	0.6 – 1.3
601-1200 DDD	33	2.6 %	130	2.0 %	1.2	0.9 – 1.8
> 1200 DDD	26	2.0 %	150	2.3 %	0.9	0.6 – 1.3
Average day dose						
0 DDD/day	1187	93.3 %	6087	93.4 %	1.0	referent
0.01-1.00 DDD/day	45	3.5 %	225	3.5 %	1.0	0.7 – 1.4
1.01-1.50 DDD/day	10	0.8 %	62	1.0 %	0.9	0.5 – 1.6
> 1.5 DDD/day	30	2.4 %	146	2.2 %	1.0	0.7 – 1.5
USE OF AR BLOCKERS [d]						
Users versus non-users	n=1217		n=6235			
non-exposed	1187	97.5 %	6087	97.6 %	1.0	referent
exposure >0.5 yr	30	2.5 %	148	2.4 %	1.0	0.7 – 1.5
Cumulative prescription duration [c]						
non-exposed	1187	97.5 %	6087	97.6 %	1.0	referent
1-750 days	20	1.6 %	70	1.1 %	1.4	0.9 – 2.1
>750 days	10	0.8 %	78	1.3 %	0.7	0.4 – 1.3
Cumulative dose						
0 DDD	1187	97.5 %	6087	97.6 %	1.0	referent
1-1000 DDD	22	1.8 %	82	1.3 %	1.3	0.8 – 2.0
> 1000 DDD	8	0.7 %	66	1.1 %	0.7	0.3 – 1.4
Average day dose						
0 DDD/day	1187	97.5 %	6087	97.6 %	1.0	referent
0.01-1.00 DDD/day	16	1.3 %	89	1.4 %	0.9	0.6 – 1.5
> 1.0 DDD/day	14	1.2 %	59	0.9 %	1.2	0.7 – 2.0

[a] Adjusted for the total number of unique medical diagnoses and the use of statins.
[b] All commercially available ACE inhibitors in The Netherlands between 1991 and 2004.
[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] All commercially available AR blockers in The Netherlands between 1991 and 2004.
OR = Odds Ratio, CI = Confidence Interval, ACE = Angiotensin-Concerting Enzyme, AR = Angiotensin Receptor.

number of ACEi and ARb users leading to limited statistical precision, especially for the stratified analyses. Another limitation is the relatively short follow-up (3 years). We decided to use only cases and controls with complete follow-up to guarantee that cases and controls were active members of the PHARMO network and thus all prescription drugs dispensed would be registered in PHARMO. Due to sample size limitations, we were not able to study the effects of drug use longer than 3 years before cutaneous melanoma. However, the length of follow-up in our study was comparable with the median follow-up in a previous study in which exposure to ACEi and ARb was significantly associated with reduced risks of basal cell carcinoma and squamous cell carcinoma. [12]

Residual confounding may have affected our findings. ACEi and ARb 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 cannot be excluded.

Common risk factors for melanomas, such as family history of melanoma, skin type, sun exposure history and socioeconomic status, are not available in PHARMO and PALGA. Therefore, we could not adjust for these factors. Skin type and family history of melanoma are, in our opinion, unlikely to affect the likelihood of prescription of ACEi and ARb. Thus, confounding by indication by these seems unlikely. Sun exposure, however, may be indirectly related to ACEi and ARb exposure because it may be associated with increased physical activity and a reduced chance of hypertension. Likewise, high social economic status is associated with increased sun exposure and may also be associated with a reduced chance of hypertension. Both these potential biases would in an underestimation of any effect of ACEi and ARb and would thus produce bias toward the null.

An additional source of residual confounding may be exposure to NSAIDs obtained as over-the-counter drugs that will not always be registered in PHARMO. However, such misclassification is likely to be equal among cases and controls; hence, bias is likely to be minimal.

Despite the limitations mentioned, we believe the results of our study with adjusted ORs near to 1.0 emphasize the possibility that ACE inhibitors and AR blockers at current dosage may not affect melanoma development.

Conclusion

In this study, the use of ACEi or ARb does not seem to protect against the development of cutaneous melanoma. However, we cannot exclude an association between ACEi and ARb exposure and an increased or decreased incidence of cutaneous melanoma.

Acknowledgement

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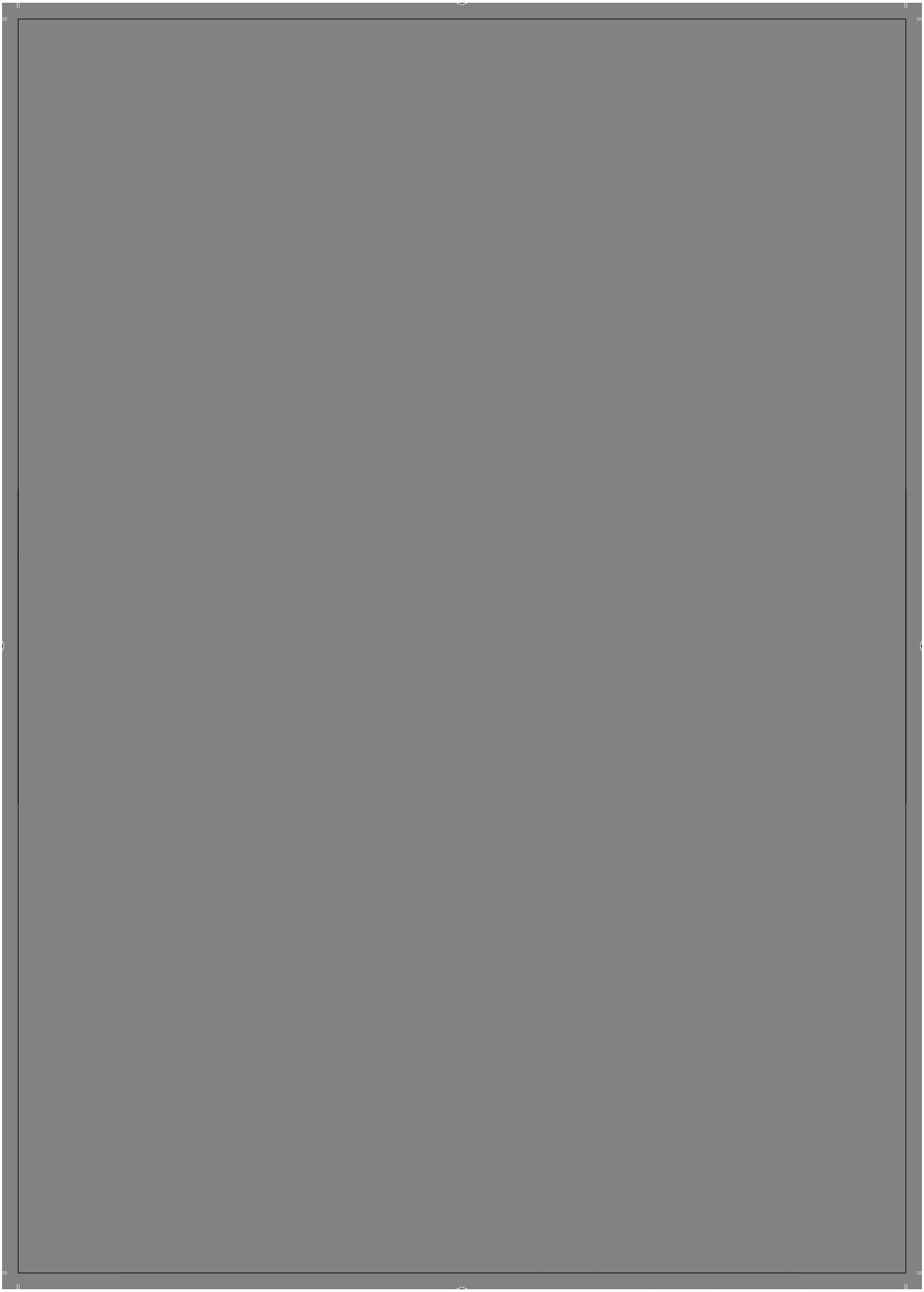

Chapter 8

Estrogens, oral contraceptives and hormonal replacement therapy, increase the incidence of cutaneous melanoma: a population-based case control study

8

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Abstract

Background: Multiple studies showed conflicting results on the association between oral contraceptive use and the development of cutaneous melanoma (CM). We investigated the association between estrogen use and CM incidence.

Patients and methods: Data from PHARMO Pharmacy database and PALGA, the pathology database in the Netherlands, were linked. Women, ≥ 18 years, with a pathology report of a primary CM between January 1st 1991 and December 14th 2004 and ≥ 3 years of follow-up prior to CM diagnosis were eligible cases. Controls were matched for age and geographic region. Multivariate logistic regression was used to calculate adjusted odds ratio (OR) and 95% confidence interval (CI) for the association between CM incidence and estrogen use, oral contraceptives (OC) and hormonal replacement therapy (HRT), separately.

Results: In total, 778 cases and 4072 controls were included. CM risk was significantly associated with estrogen use (≥ 0.5 year; adjusted OR=1.42, 95% CI: 1.19-1.69). This effect was cumulative dose-dependent (p-trend < 0.001). CM risk was also significantly associated with the use of HRT (≥ 0.5 year: OR=2.08; 95% CI: 1.37-3.14) and OC (≥ 0.5 year: OR=1.28; 95% CI: 1.06-1.54).

Conclusion: Our study suggests a cumulative dose-dependent increased risk of CM with the use of estrogens.

Introduction

The influence of estrogens on the incidence of cutaneous melanoma (CM) in women has been supported by a number of observations. Firstly, indicating the effect of estrogens on melanocyte proliferation, hyperpigmentation is a side-effect of oral contraceptive (OC) use and may also occur during pregnancy (chloasma) or with the use of hormonal replacement therapy (HRT). [1] Secondly, until the age of ~45 years, CM incidence rates exceed those in men, after which the incidence rates in men rise markedly, but level off in women. Since the incidence rates of CM in women mimic those of breast cancer, female sex steroids have been hypothesized to be involved in the development of CM in women. [2] Additionally, recent studies have demonstrated improved survival among women compared to men with CM after adjusting for demographic and tumor characteristics. [3, 4] One of the possible explanations may be influence of estrogens because it has been suggested that estrogens are associated with melanomas with a relative good prognosis such as superficial spreading melanomas. [4] In a previous study we observed that ever use of estrogens was associated with an increased incidence of CM. [5] Moreover, women with a history of breast cancer have been reported to be at higher risk of CM and vice versa. [6] Also, estrogen-binding receptors have been detected in melanomas and benign *nevi* [7].

On the basis of these observations, several epidemiological studies have investigated the association between OC use and CM development. These studies show, however, inconsistent results. About 25-30 years ago, a higher CM incidence was suggested among women using OC compared to women who never used OC in three cohort studies. [8-10] Nevertheless, subsequent (case control) studies failed to confirm a significant effect of OC on the incidence of CM. [11-15] However, a few large studies with long-term follow-up and a relatively high proportion of women having used OC for a long period of time did show a significant two- to four-fold increased likelihood to develop CM. [16-18] In a pooled analysis of 10 case-control studies Karagas *et al.* [2] observed no excess CM risk associated with OC use for 1 year or longer compared to non-users. However, these studies are limited in sample size and included selected study populations. Prior estrogen use was assessed by interviews or questionnaires, which may result in a recall bias. Moreover, no differentiation was made between OC and HRT. In this case control study, we linked a population-based pharmacy database with the national pathology database to assess the association between the incidence of CM and estrogen, OC and HRT use, separately.

Patients and methods

Setting

Data were used from the PHARMO database, containing drug-dispensing records of a defined population of more than 2 million Dutch residents representing >12% of the Dutch population. Residents are included regardless of type of health insurance or other relevant factors. [19]

The core file of the PHARMO system is a patient-roster file which includes of all patients an entry- and exitdate. To this roster file, the drug dispensing records of all pharmacies, and pathology records are linked on a patient-centric level. Since most individuals designate a single pharmacy in The Netherlands, dispensing histories are virtually complete. [20] The computerized drug-dispensing histories contain all dispensed prescriptions and include 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 histo- and cytopathology, using a variation of a reliable probabilistic algorithm. [21] PALGA contains abstracts of all pathology reports with encrypted patient identification and diagnostic terms being in scope with SNOMED classification. Since 1990 the registration reached 100% participation and in 2004 over 9 million patients were archived. [22] Therefore, PALGA represents all Dutch patients and is the basis for the Netherlands Cancer Registry.

We reported our study according to the STROBE guidelines. [23]

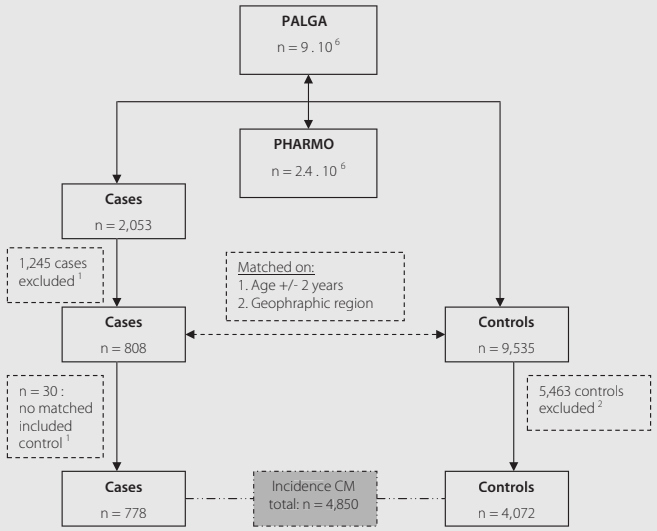
Study population

Cases had a primary CM diagnosis in PALGA from 1 January 1991 to 14 December 2004 and were also followed by PHARMO at any point in this period. End of follow-up was defined as the date of CM diagnosis (index date).

For each case all records in PALGA were read by one of two investigators (AJ, ERK). From these records, ERK and AJ extracted and recorded final diagnosis, date, anatomical location and CM subtype according to WHO classification [24] of the primary CM. To assess interobserver variation, 300 cases were randomly selected and scored by both researchers.

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

Figure 1 Flow chart study population †



[1] Cases excluded	Total	n = 1,275	100 %
1 = Entrydate missing		n=34	2.7 %
2 = Age at diagnosis < 18 yrs		n=15	1.2 %
3 = Follow up 3 yr before CM incomplete		n=810	64 %
4 = CM not pathologically confirmed		n=195	15 %
5 = Primary CM before 1991		n=59	4.6 %
6 = no cutaneous MM (i.e. eye)		n=102	8.0 %
8 = in situ CM		n=30	2.4 %
9 = no matched controls included		n=30	2.4 %

[2] Controls excluded	Total	n= 5,463	100 %
1 = Entrydate missing		n= 373	6.8 %
2 = Age at diagnosis < 18 yrs		n= 9	0.2 %
3 = Follow up 3 yr before CM incomplete		n= 5,079	93 %
4 = possible CM patient (ICD code 'CM')		n= 2	0.04 %

† All numbers presented represent the number of patients (cases or controls) involved.

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

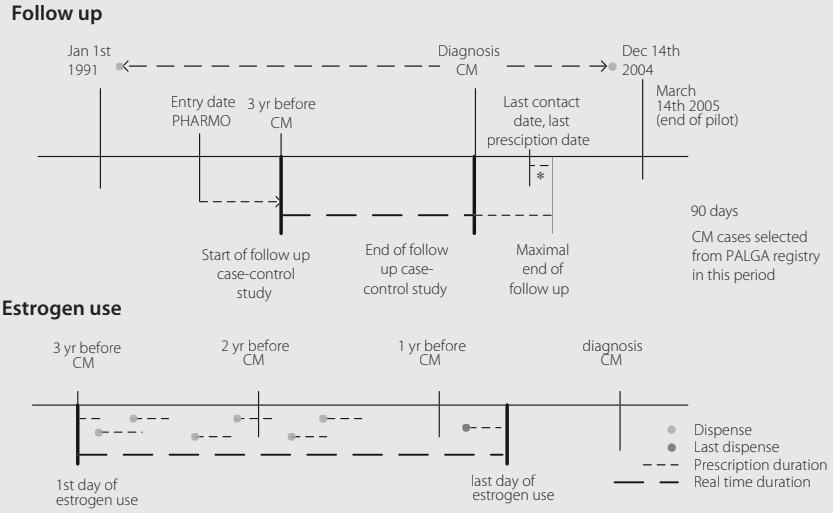
Potential controls were excluded if, in PHARMO, a date of entry was unknown, they were younger than 18 years at the index date, follow-up in the 3 years before index date was incomplete, or if they were diagnosed in PHARMO with previous CM according to the International Classification of Disease (Fig.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

Drug Exposure

Estrogen exposure was expressed in defined daily doses (DDDs) according to the WHO definitions. It was defined as the use of one or more estrogens containing formulations for at least 6 months of cumulative prescription duration in the 3 years before CM because a minimal exposure was assumed to be required for the hypothesized biologic mechanism (Fig. 2). All estrogens commercially available and approved in The Netherlands in the study period were included. Estrogen use was differentiated between OC (ATC codes: G03AAXX and G03ABXX) and HRT (ATC code: G03CAXX). For OC, daily use for 21 days and subsequently a 7 days period of non-use was assumed and therefore we divided the prescribed daily dose by 0.75 (= 21 of 28). Some OC formulations are used for 22 days with a 6-day drug-free period. However, this applied to only 6.2% of the prescriptions and the error is < 5%. Therefore, we did not correct for this.

For locally applied HRT, i.e. vaginal therapy, only dispenses with a prescribed dose corresponding with a minimal systemic exposure of 0.25 DDD/day orally were included. To further detail estrogen use, the cumulative dispensed dose and the cumulative prescribed duration were calculated (Fig 2).

Figure 2



Potential confounders

Ever use of drugs possibly related to progression and development of CM such as non-steroidal anti-inflammatory drugs (NSAIDs including COX-2-inhibitors) and statins was assessed [25]. Use of fibrates and lipid-lowering drugs other than fibrates or statins [25] was recorded, but the number of cases and controls using these drugs were too small (<1.0 %) to be used in further analysis.

To estimate health care consumption, which may affect the likelihood of CM diagnosis, the total number of unique prescriptions (ie, number of different ATC codes excluding estrogens) recorded in PHARMO in the 3 years before CM was included.

Statistical analysis

To test for statistical differences, χ^2 and Student's *t*-tests were used for categorical and continuous variables respectively. All statistical tests were performed two-sided with rejection of the null hypothesis at a *p*-value <0.05.

A multivariate logistic regression model was used to calculate adjusted odds ratio (OR) and 95% confidence interval (CI) for the association between CM incidence and estrogen use. In the multivariate model we included confounders with a *p*-value <0.10 in univariate analysis. The different estrogen variables were categorized based on tertiles among all users. We categorized HRT variables across the median because of the relative small numbers.

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

Results

Study population

In total, 2053 female subjects who were registered in PHARMO had a SNOMED code 'CM' in PALGA, of which 778 (37.9%) met the inclusion criteria (Fig. 1). Most of the potential cases were excluded because the time periods they were registered in PALGA and PHARMO did not match, or the follow-up in PHARMO in the 3 years before CM diagnosis was incomplete. The accordance in extracting the relevant information from the pathology records between the two authors was high (Kappa values >0.85). Of the 9535 controls matched on age (± 2 years) and geographical region, 4072 (42.7%) were eligible to enter the study.

Mean age of cases and controls was 53.6 and 54.6 years (p >0.05; Table 1). The number of unique prescriptions excluding estrogens was borderline significant with cases having more prescriptions than controls (8.25 versus 7.74, $p = 0.047$).

Table 1 Prior estrogen use (hormonal replacement and contraceptives) and characteristics of the study population

		Cases (778)		Controls (4072)		p-value	Adjusted OR [a]	95% CI
		n	%	n	%			
Age at diagnosis [b]	yrs	53.6 ±	16.5	54.6 ±	16.1	0.13	-	-
Total unique diagnoses [b]	number	0.62 ±	1.33	0.59 ±	1.50	0.55	-	-
Total unique prescriptions [c]	number	8.25 ±	7.37	7.74 ±	6.48	0.05	-	-
NSAIDs [d]	yes	388	49.9	1817	44.6		-	-
	no	390	50.1	2255	55.4	<0.01	-	-
Statins [d]	yes	40	5.1	265	6.5		-	-
	no	738	94.9	3807	93.5	0.15	-	-
Estrogen use [e]	non-exposed	577	74.2	3270	80.3		1.00	referent
	exposure >0.5 yr	201	25.8	802	19.7	<0.001	1.42	1.19 - 1.70
Cumulative prescription duration [d,f]	non-exposed	577	74.2	3270	80.3	[g]	1.00	referent
	1-700 days	75	9.6	279	6.9	<0.01	1.51 [h]	1.15 – 1.98
	701-1100 days	63	8.1	301	7.4	0.24	1.19 [h]	0.89 - 1.58
	>1100 days	63	8.1	222	5.5	0.001	1.61 [h]	1.20 - 2.16
Cumulative dose	0 DDD	577	74.2	3270	80.3	[g]	1.00	referent
	1-650 DDD	63	8.1	271	6.7	0.06	1.31 [h]	0.98 - 1.75
	651-1000 DDD	68	8.7	270	6.6	0.01	1.44 [h]	1.08 - 1.90
	> 1000 DDD	70	9.0	261	6.4	<0.01	1.51 [h]	1.14 – 1.99

[a] Adjusted for the total number of unique prescriptions dispensed (excluding estrogens) and the use of NSAIDs.
[b] Mean value presented ± standard deviation, tested for statistical difference with t-test, equal variances assumed.
[c] Mean value presented ± standard deviation, tested for statistical difference with t-test, equal variances not assumed.
[d] Number of cases and controls presented, tested for statistical difference with χ^2-test.
[e] Estrogen use: hormonal replacement therapy (HRT) and oral contraceptives (OC).
[f] Time interval between estimated last day of use (based on last dispense and amount dispensed) and date of diagnosis of CM. The cumulative prescription duration may exceed maximum number of days of follow up (3 years) due to overlapping dispenses.
[g] p-value for trend analysis: <0.001
[h] p-values calculated for each category of estrogen-users compared with non-users.

NSAIDs, non-steroidal anti-inflammatory drugs; OR, odds ratio; CI, confidence interval; DDD, defined daily dose.

Estrogen use

Estrogens were used for more than half a year in the study period by 25.8% of the cases and 19.7% of the controls (p <0.001; Table 1). Of estrogens used, 83.4% was ethinyloestradiol, 9.1% was oestradiol, 4.8% were conjugated estrogen, 2.0% was oestriol and 0.6% was mestranol.

Cases and controls did not differ in the average day dose of estrogens. Cases were prescribed an average estrogen day dose of 0.73 DDD/day [standard deviation (SD) 0.19] and controls were prescribed 0.75 DDD/day [SD 0.16].

In univariate analysis, estrogen use (≥ 0.5 year), ever NSAID use, a cumulative prescription duration (1-700 days or >1100 days) or a cumulative dose of estrogens (651-1000 DDD or >1000 DDD) were significantly associated with the incidence of CM (p < 0.05).

After adjusting for confounding factors in a multivariate model, estrogen use (≥ 0.5 year) remained significantly associated with a higher risk of developing CM (adjusted OR = 1.42, 95% CI = 1.19-1.70).

Compared to controls, CM patients were significantly more likely to have used higher cumulative doses. Compared to female non estrogen users, women who used estrogens >1100 days were ~60% more likely to have developed a CM (adjusted OR = 1.61, 95% CI = 1.20-2.16). For the cumulative prescription duration as well as the cumulative dose, a statistically significant trend was detected (p <0.001).

Oral Contraceptives

OC was used in the study period for more than half a year by 21.5% of the cases and 17.7% of the controls. Of the oral contraceptives used, 99.3% was ethinyloestradiol and 0.7% was mestranol. In univariate analysis, OC use (≥ 0.5 year) was significantly associated with the incidence of CM (p < 0.05; Table 2). After adjusting for confounding factors in a multivariate model, OC use remained significantly associated with development of CM (adjusted OR = 1.28, 95% CI = 1.06-1.54). Compared to female non estrogen users, women who used estrogens longer than 1100 days were more likely to have developed CM (adjusted OR = 1.56, 95% CI = 1.16-2.10). Female CM patients were significantly more likely to be included in the highest category of cumulative dose than those without CM (>1000, adjusted OR = 1.44, 95% CI = 1.08-1.94, compared to 0 DDD). For the cumulative prescription duration (p-trend analysis <0.01) as well as the cumulative dose (p = 0.01), a statistically significant trend was found.

Hormonal Replacement Therapy

HRT was used for more than half a year in the study period by 4.2% of the cases and 2.0% of the controls (p = 0.001; Table 2). Of the HRT used, 56.9% was estradiol, 29.9% were

Table 2 Prior use of oral contraceptives (OC) or hormonal replacement therapy (HRT) in the study population

		Cases (778)		Controls (4072)			Adjusted OR [a]	95% CI
ORAL CONTRACEPTIVES		**n**	**%**	**n**	**%**	**p-value**		
Prior OC use [b]	non-exposed	611	78.5	3351	82.3		1.00	referent
	exposure >0.5 yr	167	21.5	721	17.7	*0.01*	1.28	1.06 – 1.54
Cumulative prescription duration [b,c]	0 days	611	78.5	3351	82.3	[d]	1.00	referent
	1-700 days	56	7.2	235	5.8	*0.08*	1.31 [e]	0.96 – 1.77
	701-1100 days	50	6.4	271	6.7	*0.94*	1.02 [e]	0.75 – 1.40
	>1100 days	61	7.8	215	5.3	*<0.01*	1.56 [e]	1.16 – 2.10
Cumulative dose [b]	0 DDD	611	78.5	3351	82.3	[d]	1.00	referent
	1-700 DDD	55	7.1	256	6.3	*0.29*	1.18 [e]	0.87 – 1.60
	701-1000 DDD	50	6.4	230	5.6	*0.28*	1.21 [e]	0.88 – 1.67
	>1000 DDD	62	8.0	235	5.8	*0.01*	1.44 [e]	1.08 – 1.94
HORMONAL REPLACEMENT THERAPY								
Prior HRT use [b]	non-exposed	745	95.8	3990	98.0		1.00	referent
	exposure >0.5 yr	33	4.2	82	2.0	0.001	2.08	1.37 – 3.14
Cumulative prescription duration [b,c]	0 days	745	95.8	3990	98.0	[d]	1.00	referent
	1-671 days	18	2.3	43	1.1	<0.01	2.16	1.24 – 3.78
	>671 days	15	1.9	39	1.0	0.02	1.98	1.08 – 3.62
Cumulative dose [b]	0 DDD	745	95.8	3990	98.0	[d]	1.00	referent
	1-671 DDD	18	2.3	44	1.1	<0.01	2.13	1.22 – 3.71
	>671 DDD	15	1.9	38	0.9	0.01	2.02	1.10 – 3.70

[a] Adjusted for the total number of unique prescriptions dispensed (excluding estrogens) and the use of NSAIDs.
[b] Number of cases and controls presented, tested for statistical difference with χ^2-test.
[c] Time interval between estimated last day of use (based on last dispense and amount dispensed) and date of diagnosis of CM. The cumulative prescription duration may exceed maximum number of days of follow up (3 years) due to overlapping dispenses.
[d] *p*-values calculated for each category of estrogen-users compared with non-users.
[e] *p*-value for trend analysis: ≤0.01

OC, oral contraceptive; HRT, hormonal replacement therapy; OR, odds ratio; CI, confidence interval; DDD, defined daily dose.

conjugated estrogens, 12.5% was estriol and 0.6% was ethinyloestradiol. In univariate analysis HRT use (≥ 0.5 year) and the highest categories of cumulative prescription duration and dose of HRT were significantly associated with the incidence of CM ($p < 0.05$; Table 2). After adjusting for confounding factors in a logistic multivariate model, HRT use (≥ 0.5 year) was still significantly associated with development of CM (adjusted OR = 2.08, 95% CI = 1.37-3.14). In the multivariate model, female CM patients were two folds more likely to have used HRT for a longer duration and higher cumulative doses than those without CM.

Subgroup analysis

Restricting the multivariate analysis to the effect of estrogen use (≥ 0.5 year) on the incidence of superficial spreading CM showed comparable results (adjusted OR = 1.46; 95% CI = 1.18-1.81). If the analysis was restricted to nodular CM, lentigo maligna and others, the risk estimate is slightly lower (adjusted OR = 1.12, 95% CI = 0.74-1.69).

Discussion

Estrogens – in general

Estrogen use, both OC and HRT therapy, was associated with an increased incidence of CM. Although we can only speculate about the causality based on observational studies, the significant dose-effect relationships we detected do support our hypothesis. Previous studies are not in accordance with our findings. This may be due to lower cumulative doses of estrogens being used or limited sample sizes. Early case control studies [8-10], which also supported an increased risk of CM with estrogen use, are likely to have included higher doses (doses of estrogens used have declined since the 1970's). On the based of an overall CM incidence among women in The Netherlands in 2000 of 16 per 100.000 person-years [26], a female population of 8,02 million [27] of which 20% uses estrogens and an estimated relative risk of 1.42, the crude estimate for the incidence of CM among non-users of estrogens would be 15 per 100.000 person-years and would increase to 21 per 100.000 person-years with estrogen use.

Oral Contraceptives versus Hormonal Replacement Therapy

Although the adjusted OR of the association between OC and HRT and CM are not statistically different, the difference is striking (OC: OR = 1.28, 95% CI = 1.06-1.54, HRT: OR = 2.08, 95% CI = 1.37-3.14). Theoretically, there are several important differences between OC and HRT. The age distribution differs (ie, HRT is used in post and OC in pre menopausal

women). However, both age at diagnosis as well as an multiplicative interaction term of estrogen use and age were not statistically significant in multivariate analysis. In this study, it is impossible to differentiate between the effects of the HRT and more variable or lower endogenous estrogens on development of CM. Also, HRT consist of estrogen monotherapy, whereas OC usually is a combination of an estrogen with a progestagen. No effects of progestagens on the incidence of CM have been published. However, as Dobos pointed out, very limited data are available on the progestagen effects on the biological behavior of CM. [28] In contrast to OC, which nearly always contains ethinyloestradiol, HRT mostly contains oestradiol, conjugated estrogens or oestriol. The regimen in which OC and HRT differs because HRT can be used intermittently or continuously and OC is normally used once daily for a period of 21 days/month.

Estrogens used as HRT are often applied locally. To affect CM incidence a systemic exposure is warranted, therefore, we only included HRT dispenses that were likely to result in a minimal systemic exposure of estrogens (≥0.25 DDD/day orally). Obviously, our results also do not apply for estrogens applied locally in relatively low doses (for instance vaginal therapy twice weekly).

Strengths and limitations

This is the largest case-control study exploring the effects of estrogen use on CM incidence including >750 female cases. Both cases and controls in our study were sampled from PALGA and PHARMO. These databases are general population-based and reflect the Dutch population well. [21-22] Moreover, pharmacy data are gathered prospectively avoiding recall bias. Since we had detailed drug dispensing information, we were able to study dose-effect responses, differentiate between OC and HRT, and exclude low-dosed topically applied estrogens. Confounding by indication seems unlikely because risk factors of CM do not affect the prescription of OC. It seems highly unlikely that menopausal complaints (e.g., flushing and vaginal atrophy) or causes of menopause (e.g., hysterectomy) are associated with the incidence of CM, unless estrogens are prescribed for osteoporosis. Because osteoporosis is associated with low endogenous vitamin D levels and low sun exposure, which affect CM incidence in opposite directions [29], the association between HRT use and CM development may be affected. Unfortunately, vitamin D levels and measures of life-style factors such as sun exposure were not available in the PHARMO database. To our knowledge, only one study has studied the association between estrogen use and sun exposure and demonstrated that HRT users did not differ in sun exposure compared non-users, but users were more likely to use sunbeds. [30] For OC, the association with sun (bed) exposure use has not

been documented. To minimize the ascertainment bias, the analyses were adjusted for a proxy of health care consumption (i.e. the number of unique ATC codes). We limited the study to the effects of estrogen use in the 3 years prior to CM diagnosis to not exclude too many patients. For some subgroup analyses, especially for the use of HRT, the sample sizes may be too small. Most cases were excluded because they were registered in PHARMO in a different time period.
Unfortunately, the variation in average day dose of estrogens, expressed in DDD, among users was minimal and therefore any possible associations between average day dose and CM incidence would not have been detectable in our population. Therefore we did not include average day dose in our analysis.

Conclusion

This large observational study suggests a cumulative dose-dependent increased risk of CM with the use of estrogens. In our study, women who used estrogens for more than half a year were about 40% more likely to have developed a CM than women who did not use estrogens or less than half a year (adjusted OR = 1.42, 95% CI = 1.19-1.70). A validation of our findings is warranted, preferably in a (prospective randomized) study with detailed propectively gathered information on both drug use as well as sun (bed) exposure. Moreover, more experimental research is warranted to elucidate the effects of estrogens, progestagens and gender on CM development and progresison.

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

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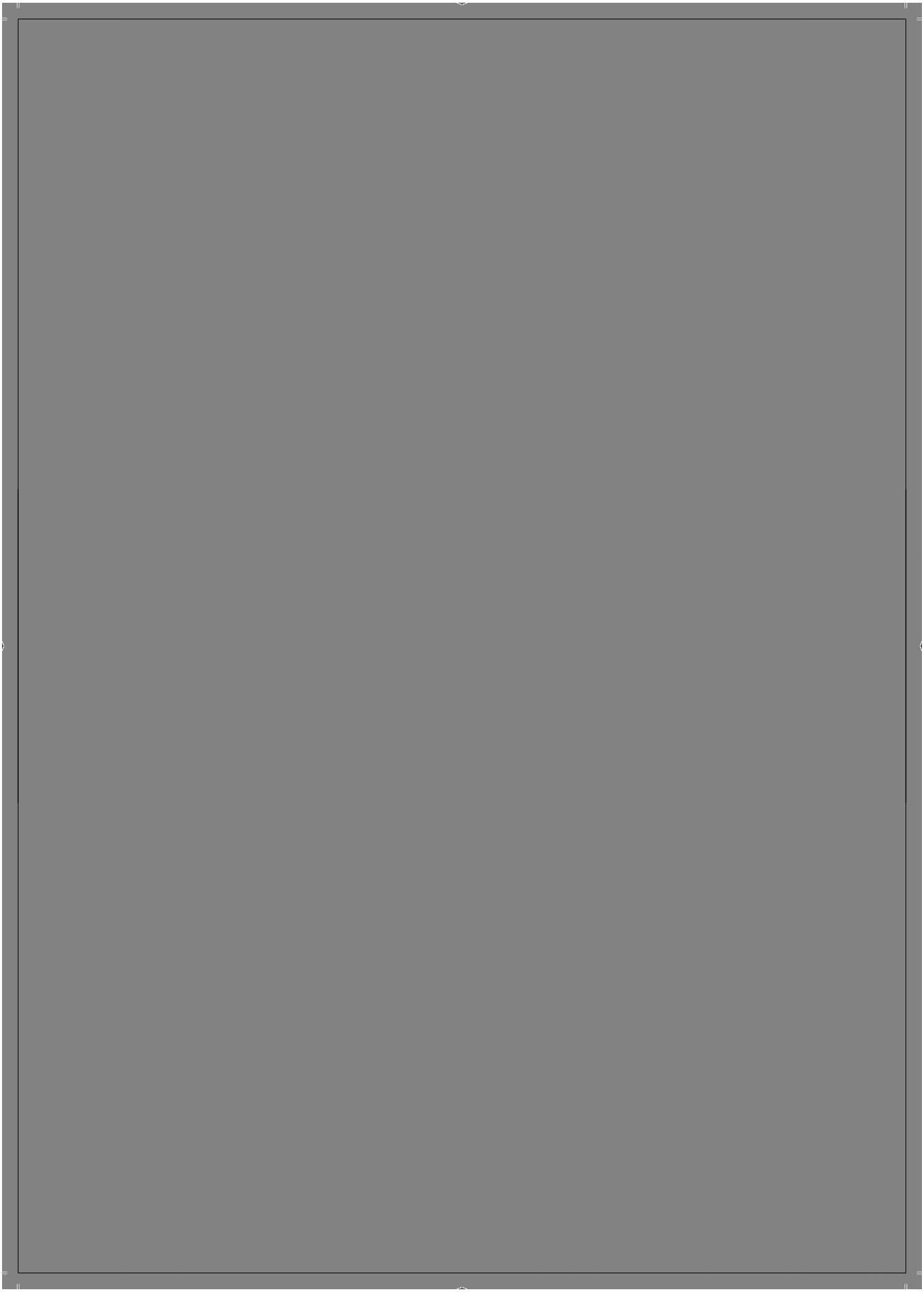

Chapter 9

Does use of estrogens decrease the Breslow thickness of melanoma of the skin?

Oral Contraceptives and Hormonal Replacement Therapy

9

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Melanoma Research 2009, 19(5), 327-332

Abstract

Background: Recently, we demonstrated there was a cumulative dose-dependent association between the use of estrogens and the incidence of melanoma (CM). This association was demonstrated for both oral contraceptives (OC) and hormonal replacement therapy (HRT). Some *in vitro* studies, however, have suggested a direct inhibitory effect on melanoma tumor growth. Therefore, the use of different types of estrogens, OC and HRT, may be associated with a decreased Breslow thickness. Consequently, the clinical impact of our previous findings may be limited. In this study, we investigate if estrogen use (≥ 0.5 year), OC or HRT, are associated with a decreased Breslow thickness.

Patients and Methods: For this study, we linked the national Dutch pathology database (PALGA) to a pharmacy database (PHARMO). Cases were women with a primary cutaneous melanoma between January 1st 1991 and December 14th 2004, aged ≥ 18 years and having ≥ 3 years of follow-up prior to diagnosis of cutaneous melanoma.

Results: In total, 687 women with melanoma were included. Univariable linear regression analysis suggested a decreased Breslow thickness with the use of OC and HRT. Statistically significant interaction was observed between age and estrogen use (p <0.01) suggesting effect modification by age. However, in stratified multivariable analyses for different age groups (< 45 years, 45-55 years, ≥ 55 years), no statistically significant associations between the use of OC or HRT and Breslow thickness were observed.

Conclusion: An association between use of OC and HRT and Breslow thickness could not be confirmed.

Introduction

Influences of estrogens on cutaneous melanoma (CM) are suggested by several observations. First, CM incidence rates in women mimic incidence rates of breast cancer. [1] Therefore, estrogens have been hypothesized to be involved in the development of CM in women. Second, women with a history of breast cancer have been reported to be at higher risk of CM and *vice versa*. [2] In addition, estrogen-binding receptors have been detected in melanomas and benign *nevi*. [3] Moreover, indicating effects on melanocyte proliferation, hyperpigmentation can occur as a side-effect of oral contraceptives (OC), during pregnancy (chloasma) or with the use of hormonal replacement therapy (HRT). [4]

Observational studies investigating the association between estrogen use and CM development are, however, conflicting. [1] Recently, in a large population-based case-control study, we demonstrated a cumulative dose-dependent association between the use of estrogens, for both OC and HRT, and the incidence of CM. [5] The clinical relevance of our findings, however, requires further study. In explanation, estrogens may specifically be associated with melanomas with a relative good prognosis such as superficial spreading melanomas. [6] More importantly, both *in vitro* and *in vivo* studies have demonstrated that estrogens may have a direct inhibitory effect on melanoma tumor growth. [7-10] It may be, therefore, that the clinical impact of estrogen effects on melanoma is more limited than would be expected. In addition, the effects of estrogens on melanomas may differ with the type of estrogen, OC or HRT, which is used. [8]

The objective of this study, therefore, is to investigate whether use of different types of estrogens, OC and HRT, is associated with a decreased Breslow thickness across women of different age groups.

Patients and methods

Setting

Data were extracted from PHARMO, a pharmacy database representing > 12% of the Dutch population who are included regardless of type of health insurance. [11] The core file of the PHARMO system is a patient-roster file that includes entry and exit dates for all patients. To this roster file, the drug dispensing records of all pharmacies, and pathology records are linked on a patient-centric level. Dispensing histories are virtually complete. [12] The drug dispensing histories contain all dispensed prescriptions and include 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 histo- and cytopathology, with a variation of a reliable probabilistic algorithm. [13] PALGA represents all Dutch patients and forms the basis for the Netherlands Cancer Registry. Abstracts of all pathology reports are recorded in PALGA, and these have encrypted patient identification and include diagnostic terms in scope with SNOMED classification. In 2004, over 9 million patients were archived. Since 1990, the registration reached 100% participation. [14]

Study population

The study population has been described previously. [15] Briefly, cases had a primary CM diagnosis in PALGA between January 1st 1991 and December 14th 2004 and were included in PHARMO. The date of CM diagnosis was considered the end of follow-up. All records from the eligible cases in PALGA were read by one of two investigators (AJ, ERK). Final diagnosis, date, anatomical body location, continuous Breslow depth, regression and CM subtype according to WHO classification were recorded. Accordance was high (kappa values >0.85) in a random sample of 300 cases. [15]
Potential cases were excluded if, in PHARMO, a date of entry was unknown, gender was unknown, or if follow-up in the three years before CM diagnosis was incomplete. In addition, patients were excluded if, in PALGA, the date of CM diagnosis was before the age of 18 or before January 1 1991, the CM was not pathologically confirmed, the primary CM was not on the skin (e.g. in the eye) or if the CM was *in situ*.

Drug Exposure

All estrogens were included that were commercially available and approved in The Netherlands during the study period. Estrogen use was differentiated between OC (anatomical therapeutic chemical (ATC) codes: G03AAXX and G03ABXX) and HRT (ATC code: G03CAXX) and was expressed in defined daily doses (DDD) according to the WHO definitions. All administration routes were included. Locally applied HRT, however, was only included if the estimated systemic exposure was comparable with at least 0.25 DDD/day orally.
Exposure was defined as the use of one or more estrogens containing formulations for at least 0.5 year in the 3 years before CM. To further detail estrogen use, we also calculated the cumulative dispensed dose and the cumulative prescribed duration in the 3 years before CM. The cumulative dispensed dose was calculated as the sum of all dispensed estrogens in DDD. The cumulative prescribed duration was calculated as the time period between first dispense and last dispense plus the estimated time period in which the dispensed estrogens of the last dispense were used.

Potential confounders

Drugs possibly related to progression and development of CM were considered potential confounders. These included Non-steroidal Anti-Inflammatory Drugs (NSAIDs including COX-2-inhibitors) and statins. [16] As a proxy for health care consumption, we included from PHARMO the total number of unique medical diagnoses (International Classification of Disease 9th revision, clinical modification; ICD9-CM) in the 3 years before CM. As a second proxy we also considered the total number of unique prescriptions (the number of different drugs used expressed as ATC codes and excluding estrogens) in these 3 years. Age at CM diagnosis, calendar year of diagnosis, pathological subtype of CM, regression and body location of the primary tumor were also tested as potential confounders.

Statistical analysis

To test for statistical differences, χ^2 and Student's *t*-tests were used for categorical and continuous variables respectively. All statistical tests were performed two-sided with rejection of the null hypothesis at $p < 0.05$.

Multiple linear regression, using continuous log transformed Breslow thickness as dependent variable, was used to estimate the effect of estrogen use on local CM progression (adjusted coefficients and 95% confidence interval (CI)). Since differences on log scale correlate to proportions on normal scale (i.e., log A – log B = log A/B), the results will be presented as a percentage change. This estimated percentage change in Breslow Depth can be calculated with the following formula:

$(e^{coefficient} - 1.00) \times 100\ \%$

Estrogen, OC and HRT variables were divided in categories of equal distances to ease the interpretation of the findings. Potential confounders were included in the multivariable model if they influenced the estimate by 10% or more. [17] Separate analyses will be performed for OC and HRT. To test for effect modification, interaction terms of different variables for estrogen use with age were tested in the multivariable linear model. As effect modification was present, separate analyses will be presented for different age groups (< 45 years, 45-55 years, ≥ 55 years; i.e., premenopausal, menopausal and postmenopausal age groups).

All statistical analyses were performed using SPSS 16.0 (SPSS Inc. Chicago, IL). This publication is reported according to the STROBE guidelines. [18]

Results

Study Population and Melanoma Characteristics

Of the 778 included female cases in the baseline study [15], 91 were excluded because of missing data on Breslow thickness or body location of the primary melanoma. The demographics are summarized in Table 1. Mean age of eligible women was 53.3 years [standard deviation (SD) 16.6].

Table 1 Demographics of the study population and melanoma characteristics

	Cases (n = 687)
Age at diagnosis	
mean ± standard deviation	53.3 ± 16.6 yr
range	18 yr - 94 yr
Drug use *(n, %)*	
estrogen users [a]	178 (26 %)
OC users [a]	151 (22 %)
HRT users [a]	26 (3.8 %)
statin users [b]	39 (5.7 %)
NSAID users [b]	335 (49 %)
Melanomas	
Breslow thickness	
median	0.90 mm
interquartile range	0.5 mm - 1.8 mm
Body location *(n, %)*	
extremities	393 (57 %)
trunk	220 (32 %)
head & neck	74 (11 %)
Pathological subtype [c] *(n, %)*	
superficial spreading	469 (68 %)
nodular	91 (13 %)
lentigo maligna	33 (4.8 %)
other	94 (14 %)
Regression [d] *(n, %)*	49 (7.1 %)

[a] At least 6 months of drug use.
[b] Ever drug use.
[c] Pathological CM subtype according to WHO classification.
[d] Regression mentioned in the pathologist report.
OC = oral contraceptive, HRT = hormonal replacement therapy, NSAID = non-steroidal anti-inflammatory agent, CM = cutaneous melanoma, WHO = world health organization.

Table 2 Pathological subtype and Breslow depth in different age groups

	Age group (Total: n= 687)					
	< 45 yrs		45 – 55 yrs		≥ 55 yrs	
Breslow thickness[a]	OC users (n=118)	Non-users (n=119)	OC/HRT users (n=33)	Non-users (n=101)	HRT users (n=19)	Non-users (n=289)
< 1.01 mm	78 (66%)	75 (63%)	21 (64%)	63 (62%)	16 (84%)	123 (43%)
1.01 – 2.0 mm	22 (19%)	30 (25%)	9 (27%)	18 (18%)	0 (0%)	80 (28%)
2.01 – 4.0 mm	14 (12%)	12 (10%)	2 (6%)	16 (16%)	2 (11%)	56 (56%)
≥ 4.01 mm	4 (3%)	2 (2%)	1 (3%)	4 (4%)	1 (5%)	30 (10%)
Pathological subtype[b]						
superficial spreading	100 (85%)	92 (77%)	25 (76%)	71 (70%)	13 (68%)	161 (56%)
nodular	7 (6%)	12 (10%)	4 (12%)	11 (11%)	1 (5%)	56 (19%)
lentigo maligna	0 (0%)	1 (0.8%)	0 (0%)	2 (2%)	2 (11%)	28 (10%)
other	11 (9%)	14 (12%)	4 (12%)	17 (17%)	3 (16%)	44 (15%)

[a] Breslow thickness in AJCC categories.
[b] Pathological CM subtype according to WHO classification.

CM = cutaneous melanoma, WHO = world health organization.

The melanoma characteristics are summarized in Table 1. Breslow thickness was non-normally distributed and therefore log-transformed. The distribution of Breslow thickness in AJCC categories was: 0-1 mm: 381 (56%), 1.01-2 mm: 161 (23%), 2.01-4 mm: 103 (15%) and > 4 mm: 42 (6%). In Table 2, the Breslow thickness in AJCC categories and pathological subtype of melanoma are summarized for different women < 45 years, 45-55 years and ≥ 55 years of age, separately.

Estrogen use

Estrogens (OC and/or HRT) were used by 178 women (26%) for more than half a year in the 3 years before diagnosis of CM. Of the estrogens used, 78% was ethinylestradiol. Cases using estrogens were prescribed an average estrogen day dose of 0.94 DDD per day [SD 0.21]. The results of univariable analysis are presented in Table 3.

Table 3 Univariable linear regression on log-transformed Breslow thickness and estrogen use

Variables	Coefficient	95% CI	*p*	Change in independent variable	Estimated % Change in Mean Breslow[a]	95% CI
Demographics						
Age at diagnosis	0.116	0.078 to 0.154	<0.001	10 years	12.3	8.1 to 16.6
Calendar year of diagnosis	-0.001	-0.021 to 0.018	0.89	1 year	-0.1	-2.1 to 1.8
Total unique diagnoses	0.064	0.017 to 0.111	0.01	1 diagnosis	6.6	1.7 to 11.7
Total unique prescriptions	0.008	-0.002 to 0.018	0.13	1 prescription	0.8	-0.2 to 1.8
Tumor characteristics						
Type of melanoma						
Superficial Spreading Melanoma	*reference*	*reference*	-	-	-	-
Nodular Melanoma	1.172	1.002 to 1.342	<0.001	*vs. reference*	223	172 to 283
Lentigo Maligna Melanoma	-0.082	-0.349 to 0.185	0.55	*vs. reference*	-7.8	-29.5 to 20.3
Other subtypes	0.338	0.171 to 0.506	<0.001	*vs. reference*	40.2	18.6 to 65.9
Location of primary tumor						
Extremities	*reference*	*reference*	-	-	-	-
Trunk	-0.081	-0.222 to 0.060	0.26	*vs. reference*	-7.8	-20.0 to 6.2
Head & Neck	0.092	-0.120 to 0.305	0.39	*vs. reference*	9.6	-11.3 to 35.7
Regression of primary tumor	-0.223	-0.470 to 0.025	0.08	No regression/regression	-20.0	-37.5 to 2.5

Drug use						
NSAIDs [b]	0.024	-0.104 to 0.152	0.71	non-user/user	2.4	-9.9 to 16.4
Statins [c]	0.163	-0.113 to 0.493	0.25	non-user/user	17.7	-10.7 to 63.7
Estrogen use [c]	-0.276	-0.421 to -0.132	<0.001	non-user/user	-24.1	-34.4 to -12.4
Oral Contraceptives [c]	-0.256	-0.409 to -0.103	0.001	non-user/user	-22.6	-33.6 to -9.8
Hormonal Replacement Therapy [c]	-0.293	-0.627 to 0.041	0.09	non-user/user	-25.4	-46.6 to 4.2

[a] Since differences on log scale correlate to proportions on normal scale (i.e., log A – log B = log A/B), the Estimated % Change in Breslow Depth is calculated as $(e^{coefficient} - 1.00) \times 100\ \%$.
[b] NSAID use was defined as ever use in the 3 years before CM
[c] Estrogen use (HRT or OC), HRT use, OC use and statin use defined as use ≥ 0.5 year in the 3 years before CM.

CI = confidence interval, NSAID = non-steroidal anti-inflammatory agent, OC = oral contraceptive, HRT = hormonal replacement therapy, CM = cutaneous melanoma.

Oral Contraceptives (OC) and Hormonal Replacement Therapy (HRT)

OC were used in the study period for more than half a year by 22% of the cases. Of the OC used, 99% was ethinylestradiol. During the study period, 3.8% of the cases used HRT (≥ 0.5 year). Of the HRT used, 57% was estradiol and 33% were conjugated estrogens.

Multivariable analysis

We estimated the effect of estrogen use, OC or HRT on Breslow depth of CM with multiple linear regression, using continuous log transformed Breslow thickness as dependent variable. If an interaction term between age and estrogen use was added to the model, this resulted in a statistically significant term in the model suggesting effect modification by age (p<0.01). For this study, we therefore subsequently stratified all analyses for different age groups (< 45 years, 45-55 years, ≥ 55 years; i.e., premenopausal, menopausal and postmenopausal age groups) (Table 4).

Women younger than 45 years

Among women aged younger than 45 years, use of OC (≥ 0.5 year) was not associated with a clinically relevant decrease in Breslow thickness after adjusting for age at diagnosis, pathological CM subtype and the total number of different diagnosis in the 3 years before CM (–1.4%; 95% CI= –18.5 to 19.5%). There was also no statistically significant effect of OC with increasing cumulative prescription duration or increasing cumulative dose (respectively 1.2%; 95% CI: –6.9 to 10.1% and 0.0%; 95% CI: –7.9 to 8.5%).

Table 4 Multivariable linear regression on log-transformed Breslow thickness and estrogen use for different age groups

Variables	Coefficient [a]	95% CI	*p*	Change in independent variable	Estimated % Change in Mean Breslow	95% CI
WOMEN AGED < 45 YEARS [b] Total n=237; OC users n=118						
Estrogen use for at least 0.5 year	- 0.014	- 0.205 to 0.178	0.89	Yes/No	-1.4	-18.5 to 19.5
Cumulative duration of prescriptions	0.012	- 0.071 to 0.096	0.77	1.5 years	1.2	-6.9 to 10.1
Cumulative estrogen dose	- 0.00009	- 0.082 to 0.082	0.99	500 DDD	0.0	-7.9 to 8.5
WOMEN AGED 45-55 YEARS Total n=134; Estrogen users n=33 (26 OC users, 6 HRT users and 1 used both)						
Estrogen use for at least 0.5 year	- 0.196	- 0.488 to 0.096	0.19	Yes/No	-17.8	-38.6 to 10.1
Cumulative duration of prescriptions	- 0.087	- 0.210 to 0.036	0.17	1.5 years	-8.3	-18.9 to 3.7
Cumulative estrogen dose	- 0.074	- 0.197 to 0.048	0.23	500 DDD	-7.1	-17.9 to 4.9
WOMEN AGED ≥ 55 YEARS [c] Total n=308; HRT users n=19						
Estrogen use for at least 0.5 year	- 0.165	- 0.529 to 0.199	0.37	Yes/No	-15.2	-41.1 to 22.0
Cumulative duration of prescriptions	- 0.067	- 0.256 to 0.131	0.51	1.5 years	-6.5	-22.6 to 14.0
Cumulative estrogen dose	- 0.050	- 0.222 to 0.122	0.57	500 DDD	-4.9	-19.9 to 13.0

[a] Adjusted for age, pathological subtype of CM and total unique diagnoses in the 3 year before CM.
[b] Users of Hormonal Replacement Therapy excluded (i.e., post menopausal women excluded).
[c] Users of Oral Contraceptives excluded (i.e., pre menopausal women excluded).

CI = confidence interval, OC = oral contraceptive, HRT = hormonal replacement therapy, CM = cutaneous melanoma.

Women aged 45 to 55 years

In multiple linear regression analysis adjusting for the confounders mentioned before, use of OC or HRT (≥ 0.5 year) among women aged 45 tot 55 years was associated with an average decrease in Breslow depth of 17.8 percent (95% CI= –38.6 to 10.1%). This association, however, did not reach statistical significance. In accordance, cumulative prescription duration and dose of OC or HRT did not statistical significantly affect melanoma thickness (respectively –8.3%; 95% CI: –18.9 to 3.7% and –7.1%; 95% CI: –17.9 to 4.9%).

Women 55 years and older

Among women aged 55 years and older, use of HRT (≥ 0.5 year) after adjusting for relevant confounders was associated with a statistically non-significant decrease in Breslow thickness of 15.2% (95% CI= –41.1 to 22.0%). The results for increasing cumulative prescription duration of HRT or increasing cumulative dose of HRT were similar (respectively –6.5%; 95% CI: –22.6 to 14.0% and –4.9%; 95% CI: –19.9 to 13.0%).

Discussion

The results of this study could not confirm that the use of OC or HRT (> 0.5 year in the 3 years before CM) has a significant influence on Breslow thickness of CM. However, the association between estrogen use and Breslow thickness of CM seems to be modified by age (Table 2 and 4). Thus, also because of limited sample size, we cannot exclude decreased Breslow depth of CM among patients aged 55 years and older and using HRT (i.e. possible type II error due to lack of power in the subgroup analyses). Moreover, because of collinearity, it is not possible to differentiate between effect modifying by age or different effects of ethinylestradiol (OC) and estrogens used in HRT (estradiol, conjugated estrogens). In explanation, synthetic estrogens may have no effect on Breslow depth whereas natural estrogens do or estrogens might only have effects on Breslow depth in postmenopausal women.

Nevertheless, the observed effects may be caused by an increased incidence of thin melanomas in this population instead of growth inhibition of CM. [5] Thus, the observed effects could also support a specific increase in the incidence of superficial spreading melanomas.

This nested case-control study is large and population-based. Pharmacy data are gathered prospectively, and therefore avoid recall bias. As we had detailed drug-dispensing information, we were able to study dose-effect responses, differentiate between OC and HRT, and could also exclude low-dosed topically applied estrogens.

Although we corrected for the pathological subtypes, we cannot exclude residual confounding. PHARMO and PALGA data, for instance, do not include data on most risk factors of (thick) melanomas such as family history of melanoma, skin type and other phenotypic characteristics, sun exposure history or socioeconomic status. As these factors are unlikely to affect the likelihood of estrogen prescription, confounding by indication seems unlikely, except possibly socioeconomic status. In addition, there is no biologic basis to assume that menopausal complaints (e.g., flushing and vaginal atrophy) or possible causes of menopause (e.g., hysterectomy) are associated with the Breslow depth of melanomas of the skin.

Conclusion

Our study does not show a statistically significant association between the use of oral contraceptives or hormonal replacement therapy and the Breslow thickness of cutaneous melanoma. However, we cannot exclude decreased Breslow thickness among women using estrogens, especially among older women.

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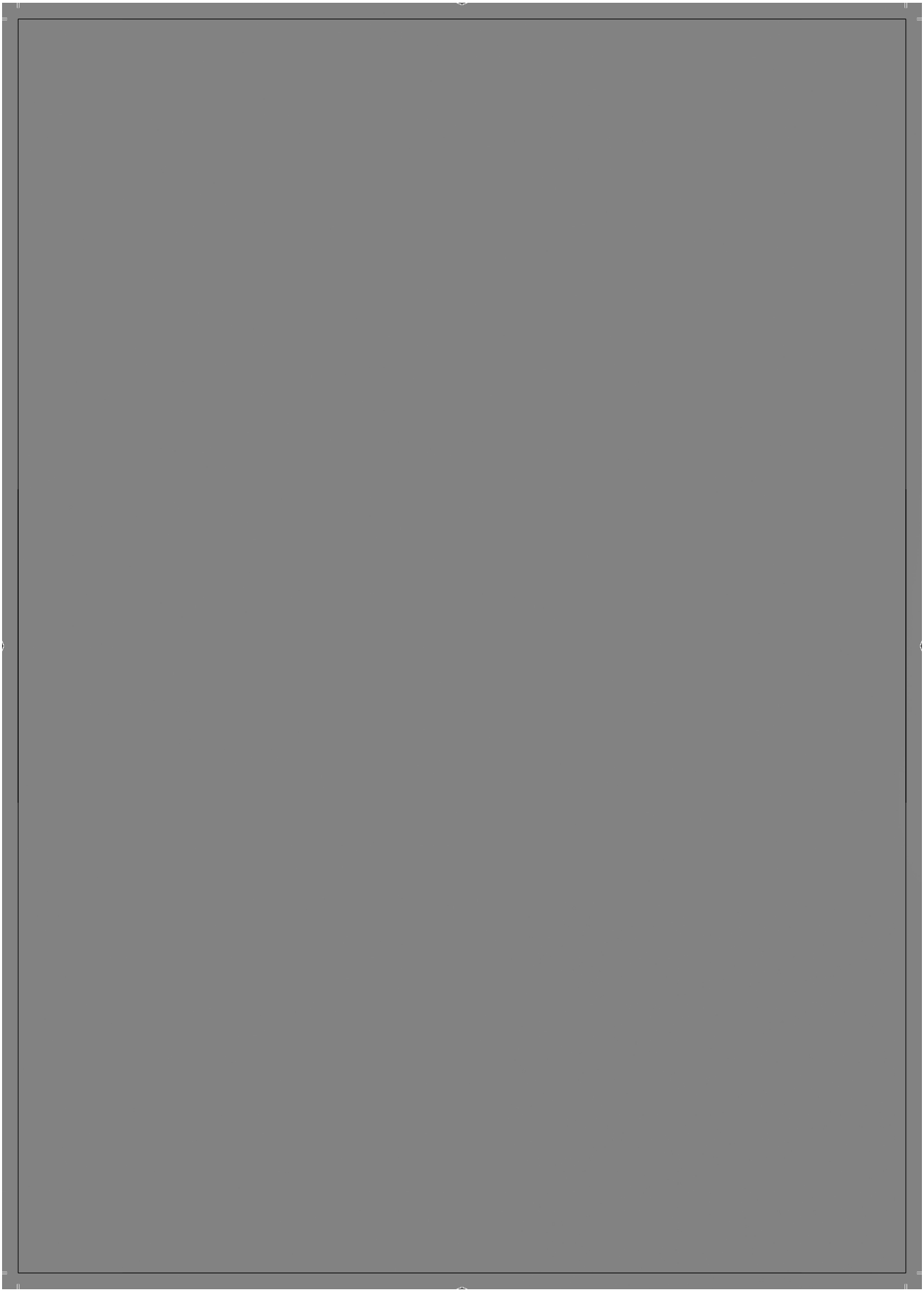

Chapter 10

General discussion, future perspectives and conclusions

10

General discussion, future perspectives and conclusions

Epidemiology of melanoma

Cutaneous melanoma (CM) in general is a growing health problem in Caucasian populations. Fortunately, the majority of cases are usually diagnosed at an early stage of the disease, that is, while the disease is still confined to the local site. Prognosis in these melanoma patients is favorable with 5-year relative survival proportions reported to be as high as 90% for women and 81% for men in The Netherlands [1]. Nevertheless, CM incidence is among the top ten of leading cancer sites among both sexes in both the United States (US) [2] and in North-western European countries [3,4]. After surgical removal of the tumor, the majority of patients will survive CM, but will remain at risk of developing regional or distant metastases for many years. [5] This risk is low but extends over prolonged periods of time. In addition, these patients are at increased risk of new primary melanomas (~5% in 20 years). [6] As a consequence, melanoma can be considered a chronic, life-threatening disease which has a significant impact on the quality of life of these patients. [5] Moreover, the incidence of CM has increased rapidly over the last decades in these Caucasian populations. [2, 7] For example, in Sweden, over a 20-year period between 1987 and 2006, CM incidence has increased with an average annual increase of 2.3% among men and 2.1% among women. [3] For The Netherlands, de Vries and colleagues have reported an annual increase in CM incidence of 2.2% in women and 3.3% in men over a 10-year period between 1989 and 1998. [7] Although a 3% annual increase may not seem large, it increases exponentially to al ready 34% in just one decade (1.03^{10}). In contrast with US data reported by the American Cancer Society indicating that CM incidence has been stable in the US since 2000 [2], we show in this thesis (**chapter 2**) that CM incidence is still significantly increasing among both sexes in The Netherlands (men: EAPC = 4.4%, 95% CI = 3.9% to 4.9%; women: EAPC = 3.6%, 95% CI = 2.9% to 4.2%). Between 1989 and 2006, the increase in CM incidence did not change (join point analysis) in The Netherlands.

Risk factors for CM include a history of sunburns, especially in childhood, high chronic sun exposure, advanced age, prior melanoma, a family history of melanoma, presence of clinical atypical nevi, and phenotypic traits, such as fair skin type, freckles, light eye color and photosensitivity. Family history of melanoma and prior melanoma are likely to be surrogate markers for genetic risk factors. In the last two decades, melanoma research has focused on finding such genetic factors. Part of the familial clustering can be explained by rare mutations in CDKN2A (encoding p16INK4a and p14ARF) and

CDK4 which are high-penetrance genes. [8,9] In addition, some low-penetrance factors contributing to melanoma susceptibility, such as single-nucleotide polymorphisms in or near MC1R, ASIP, TYR and TYRP1, have been identified. These genes determine well-established melanoma risk factors as hair and skin pigmentation, but their exact role in melanoma development remains unclear. [10] As the majority of familial cases remain unaccounted for, one may expect future additional advances in revealing genetic susceptibility genes. An individual's mutational status of such genes can in the future be used to develop personalized surveillance and prevention measures.

While the incidence of CM has increased over the last decades, mortality rates of CM seem to have stabilized or even slightly decreased. [11] In addition, CM patients are relatively young at diagnosis. [12-14] Consequently, the total burden of CM is expected to have increased in these populations. However, recent estimates of the burden of CM, other than just incidence and mortality rates, are sparse. Moreover, in most studies only a small number of the possible different measures[1] of the burden of melanoma have been compared. [12,13,15,16] Estimates of the burden of melanoma within the Dutch population were not available. Data from the Belgium National Cancer Registry (1987-1992), however, have been published. These data showed that the years of life lost per death (average years of life lost, AYLL) was 8.1 years for men and 6.3 years for women prior to the age of 65 years. [13] Since then, the incidence of CM has increased and, moreover, life expectancy will also be affected beyond the age of 65 years. Therefore, these data are likely to underestimate the current burden of CM in the Dutch population. Indeed, in **chapter 3**, we demonstrated that the total burden of CM has accumulated in The Netherlands and that the AYLL in 2002-2206 was 17.7 and 20.4 years for men and women, respectively.
By estimating a series of different measures of burden, we determined the burden of CM to the Dutch population in 5-year periods between 1989 and 2006. These measures of burden were: cumulative incidence rates, cumulative mortality rates, number of years of life lost (YLL), average number of years of life lost (AYLL; the number of years of life lost per death; YLL/deaths), number years of life lost to disability (YLD), the number of years of life lived with disease (YLWD),the average number of years lived with disability (AYLD), and the average number of years lived with disease (AYLWD) by Dutch CM patients.

1 incidence & mortality rate, prevalence, number of years of life lost (YLL), average number of years of life lost (AYLL; the number of years of life lost per death; YLL/deaths), number years of life lost to disability (YLD), the number of years of life lived with disease (YLWD),the average number of years lived with disability (AYLD), and the average number of years lived with disease (AYLWD).

The incidence of melanoma almost doubled between 1989 and 2006 (cumulative incidence rate increased from 1.0-1.3% to 2.0-2.1%). Likewise, the cumulative mortality rates also doubled up to 0.61 for males and up to 0.40% for females. Suprinsingly, age at diagnosis of melanoma increased over time.

On average, patients lived 21.5-28.4 years with a melanoma diagnosis and melanoma resulted in a loss of about 18-20 years before the age of 95 for those that died of their melanoma. Including all patients diagnosed with a melanoma, not only those that die from it, the average life loss is about 3 years.

Overall, the burden of melanoma to society increased rapidly between 1989 and 2006.

As a consequence of the high burden of melanoma, some argue that melanoma is among the 'Cinderella cancer types'. For example, Burnet and colleagues compared the average years of life lost due to 17 different cancer types with the research funds spent on these cancer types by the National Cancer Research Institute of the UK. This led to the conclusion that, based on this ratio, melanoma as well as tumors of the CNS, kidney and cervix would merit higher research funds. [16] Likewise, if one would define ranking not solely on the incident numbers or the estimated number of deaths due to a certain cancer type, but on a more detailed measure of the burden to the population, melanoma would most likely merit a higher ranking. [13] This disparity is, in part, due to the fact that relatively young people are affected by melanoma as compared to other malignancies, but, ironically, also due to the relatively favorable survival of most melanoma patients. Nevertheless, metastasis risk for CM patients is prolonged, effective treatment options are limited once (multiple) positive lymph nodes, skin metastasis or organ metastases have developed. In addition, CM incidence increases and patients with prior melanoma are at higher risk of a second primary melanoma. Therefore, in countries with high and increasing CM incidence, future health-care planning for melanoma care and surveillance is of great importance.

Although prognosis is favorable for the majority of CM patients, for some subgroups of melanoma patients, prognosis is poor. For example, patients with advanced stages of CM at diagnosis, such as regionally spread disease, have a dismal prognosis. In US data from 2008, CM patients with regional spread had a 5-year relative survival of 65.2% (versus 98.5% for CM local disease). [2] With further spread of the disease, that is, if distant metastasis has occurred, no effective treatment options are available [17] and the 5-year survival proportion even drops to 15.3%. [2]

Likewise, prognosis for patients with more rare subtypes of melanoma, such as acral lentiginous melanoma (ALM) or extracutaneous melanoma (ECM), is generally worse as compared to CM patients. [18,19] With data from the Surveillance Epidemiology

and End Results (SEER) dataset in the US, Bradford *et al.* recently estimated 5-year survival for ALM patients and CM patients to be 80.3% compared to 91.3% for CM patients. [18] However, for ECM subsites, reliable and recent incidence and survival estimates, for example, based on well-described national population-based databases or geographic regions, are largely lacking. Available European data are either outdated or concerned hospital-based series. [20,21] For the US, recent incidence data on ECM are available. Unfortunately, however, survival estimates and trends in the incidence of ECM were not reported. [22]

In **chapter 2**, we demonstrate that, in The Netherlands, 6.4% of all primary melanomas between 2003 and 2006 were ECM. In addition, we showed that, within the Dutch population between 1989 and 2006, five-year relative survival proportions of ECM patients were indeed worse in comparison with CM patients. Ocular melanoma was shown to be the most frequent subsite of ECM, and had the best survival. Five-year relative survival for ocular melanoma was 74% which was significantly less than the estimated 5-year relative survival for CM patients (86%; **chapter 2**). Mucosal melanomas were diagnosed less frequently, but survival of patients with this type of melanomas was dismal. Five-year relative survival for mucosal melanomas ranged from 15% for anorectal and esophageal melanomas combined to 40% for vulvar melanomas.

Incidence rates of ECM subsites with sufficient numbers, such as all mucosal melanomas, those of the ear-nose-throat region, and vulvar melanomas, did not show statistically significant trends in time (join point analyses and EAPC estimates). In contrast, CM incidence has significantly increased in the same time period among both sexes (men: EAPC = 4.4%, 95% CI = 3.9% to 4.9%; women: EAPC = 3.6%, 95% CI = 2.9% to 4.2%; **chapter 2**).

The increase in CM incidence is often assumed to be related to increased sun exposure (during childhood) and increased awareness. As the majority of ECM are not exposed to direct ultraviolet light and often not visible for the patient, one may postulate a lack of similar time trends in ECM incidence. Indeed, we did not demonstrate such trends. However, due to low incident numbers, the confidence intervals of the EAPC were wide for some subsites. Therefore, we cannot prove that time trends in the incidence of ECM subsites were significantly different from the time trends in CM incidence. Moreover, it is statistically impossible to prove a lack of association or, as Carl Sagan formulated, 'Absence of evidence is not evidence of absence'.

Nevertheless, differences in the demographics of affected patients, and in clinico-pathologic and molecular aspects, such as presence of c-KIT mutations, do suggest different pathways in the development of ECM as compared to CM. For example, a large proportion of CM lesions contain a BRAF- or NRAS-mutation [23], whereas 39%

of mucosal, 36% of acral, and 28% of melanomas on chronically sun-damaged skin harbor mutations and/or copy number variants of receptor tyrosine kinase KIT. [24] These differences, next to late diagnosis, may explain clinical heterogeneity, poor survival, diversity in melanoma biology, and response to therapy, and are likely to reflect differences in the causal pathways involved in the development of these melanoma subtypes.

Due to the rarity of ECM, incident numbers in some ECM subsites were low and, therefore, stratifying for the clinical stage of ECM at diagnosis in the survival analysis was impossible. Likewise, very refined clustering, such as separate clustering of anorectal and esophageal melanomas, was also impossible. Larger datasets, such as through Eurocare, could help in varying out such analyses and would, if present, improve chances of determining time trends in incidence and join points. However, data quality for these rare tumors may not be sufficient in some national cancer registries.

In conclusion, in this part of the thesis we have shown the incidence of CM has further increased in The Netherlands, and the total burden of CM has accumulated over the last decades. More than 5% of all invasive melanomas in The Netherlands have a extracutaneous origin, but survival of these patients is poor with 5-year relative survival proportions ranging from 74% to 15%, and the worst survival concerned mucosal melanomas.

Prevention of melanoma

A number of observations suggest a high potential benefit for the prevention of melanoma. First, CM incidence is increasing and the total burden of CM is accumulating. Second, effective treatment options for stage IV melanoma are lacking. Third, metastasis risk is prolonged over long time periods. And, most importantly, prognosis strongly depends on the stage at diagnosis.

Indeed, prevention has gained much interest in melanoma research. As mentioned before in this thesis, most of the established risk factors for melanoma are not amenable to intervention. Sun burns and sun exposure, as exceptions, are in theory amenable. Thus far, however, educational attempts and sun protection measures have not led to behavioral changes with regard to sun exposure and protection nor has the incidence of melanoma decreased or stabilized. [25-27] In a telephone survey among parents in the US, although the parents were aware of the need for sun protection for themselves and their children, many still considered a tanned skin to be

a healthy sign. Moreover, 13% of children sunburned during the past week or weekend, and 9% of their parents experienced a sunburn during the past weekend. [25] In addition, in a series of studies in the US, 87% of young adults going to the beach in 2007 were aware of a link between skin cancer/melanoma and tanning. However, knowledge about limiting tanning was seemingly concurrent with an increase in the attitude that having a tan looks better. [26] Australia, where a predominantly susceptible fair-skinned population is combined with high ambient UV radiation levels, has one of the highest skin cancer incidence and mortality rates of the world. Since the 1980s, large sun protection and awareness campaigns have been implemented in this country. [28] In spite of these enormous efforts, incidence rates, especially among individuals aged 40 years and above, have not decreased. [27]

From these studies, one can conclude that although knowledge and awareness about melanoma can be improved, we do not seem to be able to sufficiently influence (long-term) UV risk behavior. This so-called 'knowledge-behavior gap' suggest that we need to explore alternative preventive measures that will either create opportunities to succeed in changing UV risk behaviors or will avoid the need for changing these behaviors in individuals at risk. Either way, such preventive measures will need to be more acceptable to the public it aims at, and should, obviously, be effective, safe, cost-efficient, and preferably easy to implement. As other known melanoma risk factors, such as prior melanoma, family history of melanoma, large numbers of nevi, clinical atypical nevi, skin phototype, freckles, light eye color, and advanced age, are not amenable, the number of alternative options is limited.

Population-based skin cancer screening is one of the suggested possibilities. Prerequisites for screening to be appropriate have been defined by Wilson and Jungner in 1968. [29] Whether skin cancer screening meets these requirements, however, is uncertain. Arguments against screening in the general population could include that CM incidence may not be high enough, melanoma mortality is relatively low, any screening interval would probably be too long for the most aggressive melanomas, the fact that most melanomas already are diagnosed at an early stage, and diagnosis of suspected lesions may not be specific enough leading to a high number of false positives and unnecessary biopsies. Nevertheless, skin cancer screening remains much debated in literature [30], and a number of studies have focused on such an approach.

Free skin checks, often referred to as 'melanoma Monday', have been organized in both the USA [31] and countries in Europe [32]. These skin checks are successful in creating good publicity, an opportunity for education on melanoma, and sometimes a large number of the public attending. The number needed to prevent (NNP) one

melanoma, in explanation, the number of pathologically proven melanomas calculated per participant, varied from one melanoma per 110 (Belgium) [32], 277 (UK) [33] or 667 (Australia) [31] attendees. The lower NNPs resulted from studies in which high risk subpopulations were selectively invited to attend the free skin checks. In a US study that was neither randomized nor controlled, a reduced melanoma mortality was reported. [34] However, these study designs are insufficient to assess the merits of (skin) cancer screening for which a RCT design is indispensable.

In Australia, such a RCT was planned. Population screening was carried out in small Queensland towns, where some residents were selected for the screening, and compared with control towns, where mass screening was not offered. [35] This trial showed that such a population skin cancer screening program was feasible, increased melanoma awareness, and resulted in a higher number of CM diagnosed among men older than 50 years. [36] Specificity of screening for melanoma was 86%, and the positive predictive value was 2.5%. Follow-up of participants with a negative screening examination was, however, not conducted. Therefore, the number of true negative results and the true specificity is unknown. [37] Lack of true RCTs precludes true assessment of cancer screening programs as only these study designs can exclude lead time, length time and volunteer bias. Unfortunately, lack of funding, even in Australia with the highest world-wide incidence of CM, hampered the original plan to extend the work to a larger national trial which is needed to establish the overall merits of skin cancer screening.

Overall, these data suggest that skin cancer screening may not be efficient enough for a screening program aimed at the general public, but could be beneficial for selected high risk populations, such as men aged 50 years and older.

Another preventive measure, as an alternative or additional to classic sun prevention measures, would be the use of motivational interviewing by dermatologists to enhance patient motivation to reduce UV risk behaviors. Such motivational interviewing has been successfully implemented in health care settings by physicians to modify a variety of behaviors such as smoking. [38] In addition, cancer chemoprevention, more specifically melanoma chemoprevention, is another alternative option with potential to be an effective preventive measure among individuals at high risk of CM (Table 1).

Chemoprevention of melanoma

The concept of cancer chemoprevention was first described and defined by Sporn *et al.* in 1976. They defined 'cancer chemoprevention' as 'the use of natural or synthetic drugs to reverse, suppress, or prevent premalignant molecular or histological lesions from progressing to invasive cancer'. [39]

Table 1 Potential target populations for melanoma chemoprevention in a high risk strategy

Risk factor	Risk estimate		Reference
Previous invasive CM	SIR =	~ 4 - 25	[42,43]
Atypical mole syndrome	SMR =	~ 18	[44]
Invasive CM in first-degree relatives[1]	RR =	~ 2 - 10	[45,46]
Clinical atypical (dysplastic) nevi	RR (single) = RR (2-4 *nevi*) = RR (5-9 *nevi*) = RR (≥10 *nevi*) =	~ 2 ~ 7 ~ 5 ~ 12	[47]
Several large nevi (3-5 moles ≥ 3 mm in diameter on arms or lower legs)	RR =	~ 2.1 - 3.4	[46]
MC1R variants	RR =	~ 1.5 - 2.5	[48]
Red hair color	RR =	~ 2	[46]
High solar exposure in early childhood (<10 yrs)	RR / OR =	~ 2 - 4	[49]
History of severe sunburn	RR / OR =	~ 1.5 - 2.5	[46,49]
Past sunbed use at ages <35 yrs	RR =	~ 1.2	[50]
Occupation (airline crew)	SIR =	~ 2.5	[51]
Occupational chemical / toxic exposure[2]	Risk estimate =	~ 1.5 - 3.0	[52]

[1] One or more first-degree relatives (parent, sibling or child) with invasive cutaneous melanoma.
[2] These include: pesticides, polycyclic aromatic hydrocarbon (PAHs), benzene, and polychlorinated biphenyls (PCBs), trichloroethylene solvents, dioxin, and polyvinyl chloride (PVC), ionizing and non-ionizing radiation.

SIR = Standardized Incidence Ratio, SMR = Standard Morbidity Ratio, RR = Relative Risk, OR = Odds Ratio.

Agents proposed in literature for cancer chemoprevention differ both in origin and type of application. For example, macronutrients, micronutrients, such as vitamins & antioxidants, non-nutritive phytochemicals and several drugs have been suggested. In addition, for some agents, such as retinoids, both oral and topical application has been suggested. Some authors suggest that 'diet modification should be considered as a preferred preventive intervention given the low toxicity, low cost, and relative ease of implementation'. [40] However, any *a priori* statement on an agent's safety based on its origin is premature. For any agent, irrespective of their origin, the first requirement should be efficacy. In addition, the safety of a substance as a cancer chemopreventive agent can only be assessed after the target population and effective dosages have been established. History has taught us that even agents that were

considered to be safe can, in fact, have serious safety issues. For example, beta carotene in a (long-term) chemoprevention trial of lung cancer has been associated with an increase rather than a reduction of the incidence of lung cancers. This unexpected toxicity, that had not been observed previously, most likely was explained by differences in drug dosing scheme, more specifically the cumulative dose, and the target population. [41]
For melanoma chemoprevention, several potential high risk populations can be considered. These are presented in Table 1. The level of CM risk in a target population should be high enough in order to lead to a sufficiently high NNP and to outweigh the disadvantages of long-term chemopreventive therapy. Nevertheless, simply selecting the subpopulations with the highest risk estimates may not always lead to the best results from a public health perspective.

Several strategies are available for cancer prevention. First, one can aim *primary prevention* of the initial cancer in individuals at risk, *secondary prevention* of invasive cancer in patients with premalignant conditions, or one can aim at *tertiary prevention* among cancer patients in order to prevent second primary cancers. [53] Because the absolute risk of getting a melanoma is small, tertiary cancer chemoprevention, at least as a first goal, would seem to be the most realistic as these patients would be at sufficiently high risk of developing a second invasive melanoma. Moreover, in tertiary prevention, one could select a chemopreventive agent for its (additional) potential to prevent metastasis and, thus, combine adjuvant and chemopreventive effects increasing the potential overall benefit.
Second, cancer prevention can be performed according to a *high risk strategy* or the *population strategy*. In the high risk strategy, one detects certain individuals in the population that are most susceptible to the disease, and aims preventive interventions at these high risk individuals. In contrast with the population strategy, where one attempts to control the determinants of incidence in order to lower the overall risk of the total population. [54] In explanation, sun protection campaigns to change awareness and UV risk behavior of the general public are examples of the population strategy and examples of primary cancer prevention.
Advantages of population strategies include that the preventive intervention usually is radical (one attempts to eliminate the 'true cause'), all individuals at risks are aimed at and, thus, there is a large potential benefit for the population, and the intervention often is behaviorally appropriate. Disadvantages, however, include that the benefit on an individual level can be small (the so-called prevention paradox, a small risk for a large number of individuals at risk may give rise to more cases than a small number of

subjects at high risk of disease) which may lead to poor motivation of physicians and subjects. Moreover, as the potential benefit at an individual level can be small, the risk-benefit ratio, for a number of the subjects aimed at, can be worrisome. The high risk strategy, on the other hand, has the advantage that the intervention is appropriate to the individual which supports both the motivation of the physician as well as the patient, it leads to a cost-effective use of resources (intervention on a subset of the population), and the benefit-risk ratio is more favorable. [54] Nevertheless, the advantages of the population strategy, obviously, also reveal the disadvantages of the high risk strategy.
As mentioned, the limited effects of sun protection programs have stressed the importance of enhancing patient motivation in melanoma prevention which seems to be one of the key issues. Therefore, the high risk strategy (see Table 1) which supports the patient's and physician's motivation may be of interest. As most chemopreventive agents have demonstrated toxicity at some level (**chapter 4**), the high risk strategy certainly would be the choice of interest for melanoma chemoprevention.

For melanoma chemoprevention, several candidate drugs have been suggested. However, it is unclear which of these have the potential to be useful and safe. Therefore, in **chapter 4**, we carried out a systematic literature search in Medline, Embase, Web of Science and The Cochrane Library. We selected scientific papers on drug chemoprevention of cutaneous melanoma, restricted our review to drugs for which human data were available from clinical trials or observational research, and also included papers identified through cross referencing if they met these definitions. The efficiency of our literature search was relatively low (~75% of the finally included references did not emerge from the systematic literature search, and ~95% of the output of the literature search was excluded). This was probably caused by the fact that no MESH term is defined for 'chemoprevention'. Research would certainly benefit from such a MESH term.
Our systematic literature search identified 13 potential chemopreventive drug classes for CM. For 7 of these, human efficacy data were available. Consequently, we focused our review on the drug classes of NSAIDs, statins, fibrates, retinoids, imiquimod, dehydroepiandrosterone, and acetaminophen. On this subset from literature, we subsequently conducted a qualitative review.
In summary, the general conclusions from this review are that considerable preclinical evidence of efficacy as a melanoma chemopreventive drug exists for aspirin, NSAIDs, and statins, but clinical efficacy and long-term safety data with doses required for melanoma chemoprevention are still sparse. Moreover, validated preclinical models

are urgently needed to move melanoma chemoprevention forward. In future research, special attention should be paid to explore possible differential effects within a drug class, temporal dose-response relationships, and to possible synergistic or antagonistic effects. In addition, research should also focus on how to define the target populations and large randomized trials in high risk populations are required. Thus far, lack of definite data on efficacy in humans and profound long-term safety data in the required doses, preclude the use of chemopreventive drugs for melanoma in current practice. However, the use of relatively safe drugs indicated for other health effects but with additional chemoprophylactic properties in cancer development, such as low-dosed aspirin and statins, may be encouraged in people at increased risk of cancer.

Success factors for melanoma chemoprevention to be useful in patient practice will likely be: 1) little-or-no toxicity, to ensure safety, tolerability, and adherence (note: even mild but inconvenient side effects may have significant influence on adherence), 2) a sufficiently motivated target population, 3) a clear-cut definition of the high risk subpopulations at whom chemoprevention should target based upon validated prediction models, mutational status and, if possible, validated early biomarkers of invasive melanoma risk, and 4) a clear-cut definition of contraindications and predictors for individuals prone for the adverse events the chemopreventive drug may cause in order to withhold the drug from these individuals or to present additional preventive measure to them.

In **chapter 5**, we investigated the association between use of statins and the incidence of CM. In addition, the potential effects of prior statins use on Breslow's thickness at diagnosis of CM as well as effects on time to metastasis were studied.

None of the statin-related independent variables in our study consistently supports a risk reduction of statin use on the incidence of CM. Possibly, the average daily doses in our population (median: 1.3 to 2.0 DDD) are not high enough to show a chemopreventive effect. Follow-up may have been too short and adherence may have been too poor. However, in spite of several studies and (systematic) reviews published on the subject, evidence for a reduced melanoma incidence with statin use is lacking so far. [55,56]

Interestingly, our data did suggest that statin use is associated with a significantly reduced Breslow's 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's thickness of 1.8 mm, this would indicate an average reduction in the depth of the lesion of 0.35 mm with statin use. Among men this effect was even more pronounced with a reduction in Breslow's

thickness of –27.8% (95% CI = –43.7%, –7.4%, p = 0.01). Male non-statin users had a mean Breslow's thickness of 2.1 mm. Therefore, statin use for 0.5 year or more would result in a mean reduction of 0.58 mm. One could also argue that statin use among men is simply associated with earlier diagnosis of a CM lesion and not with slower progression of the CM lesion as especially male cases had a significant higher number of unique ICD diagnoses compared to male controls (0.84 versus 0.66, p = 0.02). However, if it is causal, this is an important finding since Breslow's thickness at diagnosis is one of the strongest prognostic determinants. [57, 58]

In **chapter 6** we studied potential effects on melanoma incidence associated with the exposure to non-steroidal anti-inflammatory drugs (NSAIDs), both acetylsalicylic acid (aspirin) and non-acetylsalicylic acid-NSAIDs. CM incidence was not significantly associated with ever non-ASA NSAID use (OR = 1.10, 95% CI = 0.97–1.24) or ever ASA use (OR = 0.92, 95% CI = 0.76–1.12) during the 3 years before index date. The use of larger quantities of non-ASA NSAIDs (>600 pills in 3 years) seemed to be protective for CM but did not reach significance (OR = 0.67, 95% CI = 0.36–1.23). The explanation, in part, could be the relatively short time of observation (3 years), limited sample size in this subgroup (<225 patients), and/or that non-ASA NSAIDs were administered as analgetics ('on demand' use). However, continuous low-dose use of ASAs was associated with a reduced likelihood of developing CM in women (OR = 0.54, 95% CI = 0.30–0.99) but not in men (OR = 1.01, 95% CI = 0.69–1.47). A significant trend (p=0.04) from no use, non-continuous use to continuous use of ASAs was observed in women.
In conclusion, in accordance with three large observational studies [59-61], we did not find a reduced CM incidence among overall non-ASA NSAID or ASA users. However, our results do suggest that, among women, continuous low-dosed ASA may be associated with a reduced incidence of CM in women. Gender-related differences in the pharmacokinetics and -dynamics of ASA [62,63] as well as gender-related differences in the biology of melanoma could be involved [64,65].

ACE inhibitors and angiotensin II antagonists have also been suggested as chemopreventive agents. In **chapter 7** we described an exploratory study investigating a possible etiological association between use of these agents on melanoma incidence and progression.
The use of ACEi or ARb did not seem to protect against the development of cutaneous melanoma nor was it associated with decreased Breslow's depth. However, the limited numbers of ACEi and ARb users, especially for the stratified analyses, has led to limited

statistical precision. Thus, these study results cannot exclude an association between ACEi and ARb exposure and either a (moderately) increased or decreased incidence of cutaneous melanoma. Moreover, residual confounding cannot be excluded. For example, sun exposure may be indirectly related to ACEi and ARb exposure because it may be associated with increased physical activity and a reduced chance of hypertension. Likewise, high social economic status is associated with increased sun exposure and may also be associated with a reduced chance of hypertension. Both these potential biases would in an underestimation of any effect of ACEi and ARb and would thus produce bias toward the null.

The design of the studies described in chapters 5, 6, and 7 may have several limitations because, at the time of designing these studies and often still, effective chemopreventive dosages, latency times, possible differential effects within subclasses, possible synergistic and antagonistic effects, and required temporal relationships were unknown. In retrospect, the duration of follow up prior to developing a melanoma should have been longer. In explanation, the latency time between exposure and effects on CM incidence could be substantially longer than three years. To compare, melanoma carcinogenesis may involve over 10 years. [66] Such latency times after exposure are, however, unknown, and will depend on the precise chemopreventive mechanism of action which determines the stage of development from benign nevus, dysplastic nevus, in situ melanoma until invasive cutaneous melanoma in which the agent is effective. In research practice, available data will limit study design possibilities.
Our relatively short follow-up (3 years) resulted from the decision to use only cases and controls with complete follow-up to guarantee that cases and controls were active members of the PHARMO network and, thus, all prescription drugs dispensed would be registered in PHARMO. Due to sample size limitations, we did not study the effects of drug use longer than 3 years before cutaneous melanoma. In retrospect, one may prefer to select cohorts of drug users (in explanation, statin-users, NSAID users, ASA-users, ACE and ARB users) and compare the melanoma incidence among these cohorts with a cohort of non-drug-users. Alternatively, one could compare with a cohort of users of a drug unlikely to have any chemopreventive or causative effect on melanoma. In such a design, one could perform Cox regression in order to efficiently use all available follow-up and, in addition, one could compare with melanoma incidence among both non-drug-users as well as drug users.

Currently, we cannot point out which agents are likely to be the most efficacious.

However, agents with additional major health benefits and few (long-term) adverse events, such as low-dosed aspirin, would in theory, have the best chance to result in a positive risk-benefit ratio. Nevertheless, efficacy essentially is the first important feature in this decision making process.

Ideally, effectiveness at the individual level would dictate the drug of choice in order to reach the best prediction of the benefit for the patient and, additionally, to create the best motivation among patients and physicians. As any chemopreventive effect would always demand long-term drug treatment in the order of at least 5-10 years, patient motivation is required for compliance to be achieved.

Important in this respect to point out is the fact that molecular diagnostics and new melanoma biomarkers have generated great interest in research. For example, some initiatives focus on such melanoma markers to predict prognosis or even therapeutic efficacy. Goal is to develop a more accurate, therapeutically predictive classification of human melanomas, and, in addition to select patient populations that would profit from specific therapeutic interventions. [67] Useful markers may include both classic prognostic factors such as Breslow's thickness and ulceration, as well as molecular markers indicating (new) pathophysiological melanoma subtypes. Hopefully, in a few years, we will have melanoma marker tests comparable with the tests available in some adjuvant or therapeutic settings, such as for estrogen and progesterone receptors in breast and prostate cancer or with the FDA approved assays for HER2, epidermal growth factor receptor and KIT [68]. For example, many targeted agents such as imatinib and cetuximab are effective only if their respective molecular markers are available for pharmaceutical intervention. Candidate markers for melanoma would include both validated susceptibility genes, such as mutational status of the high-penetrance genes CDKN2A, CDK4, and possibly of low-penetrance genes MC1R, ASIP, TYR and TYRP1, as they may prove to be markers for distinct melanoma subtypes, as well as molecular markers related to the mechanism of action of the (targeted) drug or chemopreventive agent to be used.

For cancer chemoprevention such biomarker strategies could be of great interest. For example, for chemoprevention among patients with a previous tumor, so-called tertiary cancer prevention, it may be interesting to be able to select the type of chemopreventive agent based on both molecular and histopathological aspects of the previous tumor, and the patient's risk factors.

If chemopreventive drug candidates could be tested for efficacy in validated melanoma models predictive for certain tumor types, for example, in a validated KIT mutated melanoma model, a BRAF melanoma model or a RAS mutated model, information would become available as to which agent is likely to be most efficacious

for which tumor type (targeted therapy). New and promising drugs in melanoma treatment, such as the BRAF inhibitor PLX4032 and the anti-CTLA4 antibody ipilimumab, as well as potential synergistic combinations (PLX 4032 combined with statins) should be tested in such models. Final goal would then be to test all melanoma patients for several biomarkers and use these biomarker results to classify their tumor (in combination with classical prognostic factors), predict prognosis, select which therapeutic interventions are indicated (excision range, sentinel node procedure, additional therapies, such as interferon, chemotherapy et cetera), to decide whether the patient would likely benefit from chemoprevention in order to prevent second primaries or to delay melanoma progression and, if so, to select the chemopreventive agent of choice. Ideally, one would select both the therapeutic interventions, adjuvant therapies as well as possible (chemo)preventive options on an individual level in such a strategy.

Before such a strategy could be developed, however, research progress is needed to (better) define available and new predictive biomarkers, to validate experimental melanoma models predicting the behavior of certain melanoma tumor types, such as a BRAF, N-RAS or c-KIT mutated melanomas, to test potential candidate chemopreventive drugs in these experimental models, to create melanoma risk prediction models, and to create prediction rules to select the chemopreventive drug which is most likely to be efficacious.

The chemopreventive drugs tested in validated experimental models should focus on those agents of a drug class that are pharmacologically and chemically most distinct and keeping in mind which agents of the drug class are most likely to result in an acceptable risk-benefit ratio. For example, as representatives of NSAIDs one should consider the distinct properties of several NSAIDs taking into account which NSAIDs score best with respect to cardiovascular risks, bleeding risks, which NSAIDs have differential pharmacologic effects, such as low-dosed aspirin, and which agents represent distinct chemical subclasses. Present experimental research has often focused on a very limited subset of NSAIDs and has included NSAIDs which have been established to have a more worrisome safety profile.

In addition, validated experimental melanoma models could be helpful in defining dose-effect relationships and temporal cause-effect relationships in melanoma chemoprevention, and in possible heterogeneity of effects between different melanoma cancer subtypes and different agents of a drug class.

Predictive models should include prognostic information that can be available at diagnosis (or shortly after) and should consider both risk factors predictive for a second melanoma, factors prognostic for metastasis risk (such as Breslow's thickness

and ulceration), molecular markers (S100, MC1R, c-KIT, BRAF, N-RAS), as well as mutational status (such as CDKN2A/p16^{INK4A} mutations, CDK4 mutations, MC1R variants). Prediction rules to select the chemopreventive drug of choice, however, may additionally include information on available patient risk factors that predict potential adverse effects of potential chemopreventive drugs (for example, risk factors for or evidence of previous ulcers or bleeding with respect to NSAID therapy).

Hormonal and gender differences in melanoma

In many of the studies presented in this thesis we were confronted with numerous gender differences in (the chemoprevention of) melanoma. For example, in **chapter 2** and **3**, gender differences in epidemiological measures of CM and ECM, such as the incidence, mortality, age at diagnosis, and several measures of the burden of melanoma were present. In **chapter 5**, statin use was associated with a reduced Breslow's depth only among men. In addition, in **chapter 6**, we demonstrated that continuous low-dosed aspirin use may be associated with a reduced incidence of CM in women, but not in men. Moreover, in the PHARMO-PALGA dataset we studied, estrogen use, both oral contraceptives (OC) and hormonal replacement therapy (HRT), was associated with an increased incidence of CM (**chapter 8**). Based upon these observational studies we can only speculate about the causality. However, the significant dose-effect relationships between estrogen use and CM incidence we detected did support our hypothesis. Nevertheless, most previous studies on estrogen use and melanoma are not in agreement with our findings. [69-71] In **chapter 9** we could not confirm an association between either OC use or HRT and Breslow's thickness.

Several issues related to the studies presented in **chapters 8 and 9** should be mentioned here. First, we studied estrogen use, either OC or HRT, regardless of whether they were used as unopposed estrogens or as combined estrogen-progestin. In breast cancer, combined preparations have demonstrated a clear risk increase, whereas only a slight increase was observed for unopposed estrogens. [72,73] Future studies of hormonal influences on CM, should therefore also study progestagenic effects, both progestin single therapy as well as in combined preparations. Second, we need to learn from the debate on the effects of HRT on coronary heart disease and breast cancer. [74] In this debate, conflicting results from observational research and randomized controlled trials were finally brought together by analyzing the data according to time since start of HRT. Similar data analysis seems indicated to study estrogen use and melanoma incidence. Such analysis methods, however, would require very large datasets with long follow up periods.

Third, procarcinogenic effects of estrogens through the ER-β receptor, the hypothesized mechanism for a potential increase in CM incidence, do seem to be realistic, at least for lung cancer, as was demonstrated in a post-hoc analysis of the Women's Health Initiative Trial. [75]

Gender differences in melanoma have also been described in other countries and datasets. For example, female gender has been demonstrated to be an independent predictor of survival in several populations of different geographical origin, such as in a German dataset [65], in The Netherlands Cancer Registry [76], in both the UK and Australia [77], and in the Sunbelt Melanoma Trial (North America) [78]. A female survival benefit was maintained after adjusting for well-established prognostic factors and, thus, is independent of Breslow's thickness, histological subtype, and tumor site. [76] Even more striking, this female survival benefit seems to disappear on ageing and is no longer present for women aged 65 years and older. [65]

Although chemopreventive studies in melanoma usually do not stratify across gender or predefine a statistical method to check for effect modification by gender, our results at least indicate that such gender differences should not be excluded in advance.

In conclusion, several findings all indicate that there must be some complex relationship between gender, possibly through hormones, and melanoma development and progression. These relationships have been studied over a long time, but these gender differences in melanoma are still not well understood. Influence of hormonal factors playing a role in these gender differences can still not be excluded. Nevertheless, the modest level of association between CM incidence and OC use that resulted from our studies is not in agreement with previous studies [69-71], and as CM risk is generally low, we can conclude there is no need to change OC prescription. Abandoning regular prescribing of HRT for uncomplicated menopausal complaints should be advocated, but for more urgent reasons than increased melanoma risk.

The power of a pharmaco-epidemiologic approach to the investigation of drug effects on a malignancy

We showed that it is feasible to study etiological research questions on unintended drug effects on a rare malignancy, such as cutaneous melanoma, with a pharmacoepidemiological approach through the linking of large national pathology and pharmacy databases. As such drugs, such as statins, are used by a relatively small proportion of the general population, gathering the required sample sizes would otherwise not have been feasible. This approach, therefore, creates research oppourtunities for many

other research questions on drugs and malignancies. For nearly all malignancies, data are well registered in The Netherlands and drug dispensing records are available for about 25% of the country within PHARMO. However, we should focuss only on research topics in which it is realistic to assume that exposure allocation is unrelated to the outcome of interest. Within this type of study, one may use proxy's of health care consumption, such as the number of different drugs an individuals uses or the number of different diagnoses registered in the LMR, to gain information on possible ascertainment bias. More importantly, the type of probabilistic linkage we used between the PHARMO database and other registries has meanwhile been validated. [79]

Although, a pharmacoepidemiological approach creates many opportunities in studying (unintended) drug effects on a malignancy, the design, collection and analysis of such studies coincides with many hurdles and pitfalls. For instance, defining the indexdates, required follow up ('follow back') periods in PHARMO in the right time relation with this indexdate, defining the exposure time window, and a minimal exposure threshold (either in minimal exposure time, minimal daydose or a minimal cumulative dose) is a complex issue. Assumptions with regard to temporal relationships between exposure and occuring events including latency times, effective chemopreventive dosages, and possible differential effects within subclasses need to be made and restrictions, sensitivity analyses or adjustments during analysis should be implemented to prevent biases. This type of research may be subject to some specific biases, such as guarantee-time bias or immortal time bias (as a consequence of exposure definition, exposed versus non-exposed seem to have a survival benefit), protopathic bias (exposure results from symptoms of the subclinical malignancy), and ascertainment bias (sampling chances differ between subgroupes; for instance individuals may not always be unsubscribed form the parmacy database if they moved out of the area).

Some of the difficulties we encountered were the lack of some therapeutic / prognostic information (metastases that were not pathologically confirmed), information available in plain text field only requiring reading and scoring of all pathological records manually (e.g., pathological details of the melanoma, such as Breslow's thickness, subtype, and anatomical location), and some technical difficulties such as the fact that PALGA is not a patient centric database necessitating linkage of each pathological record to the PHARMO records.

In conclusion, linking large national databases such as PHARMO and PALGA creates many research opportunities and may enable researches to study rare (adverse) events caused by drugs used in the (general) population. Improvements to large national

databases (in-hospital drug use, standardization of diagnostic information in electronic records) such as PHARMO and PALGA will further expand such research possibilities.

Conclusions

CM is a growing health problem in Caucasian populations. We showed that CM incidence is still significantly increasing among both sexes in The Netherlands. Fortunately, prognosis is often favorable. Nevertheless, for ECM cases and advanced stages of CM, prognosis is less favorable. ECM compromised 6.4% of all primary melanomas. Five-year relative survival proportions for patients with ECM ranged from 74% for ocular melanomas until 15-40% for mucosal melanomas. Although, CM incidence continued to accumulate, we did not demonstrate such time trends for ECM incidence. Overall, the total burden of melanoma is increasing in The Netherlands.

Melanoma prevention has focused on education and sun protection measures. Thusfar, this has not led to behavioral changes or to a decreased or stabilized melanoma incidence. Cancer chemoprevention could be an alternative approach in which an agent is used to reverse, suppress, or prevent premalignant molecular or histological lesions from progressing to invasive cancer. Considerable preclinical evidence of efficacy is available in literature for aspirin, NSAIDs, and statins as melanoma chemopreventive drugs. However, lack of definite data on efficacy in humans and profound long-term safety data in the required doses, preclude the use of chemopreventive drugs for melanoma in current practice.

Investigating unintended drug effects in a (rare)malignancy by a pharmacoepidemiological approach is feasible, can be validated, and may enable studies that would otherwise not have been feasible.

None of the statin-related independent variables in our study consistently supports a risk reduction of statin use on the incidence of CM. However, our data did suggest that statin use is associated with a significantly reduced Breslow's thickness at diagnosis. This effect was even more pronounced among men and, if causally related, is an important finding as Breslow's thickness at diagnosis is one of the strongest prognostic determinants.

Among users of non-steroidal anti-inflammatory drugs (NSAIDs), both acetylsalicylic acid (aspirin, ASA) and non-acetylsalicylic acid-NSAIDs, we did not find a reduced CM incidence. However, our results do suggest that, among women, continuous low-dosed ASA may be associated with a reduced incidence of CM in women.

The use of ACE inhibitors (ACEi) or angiotensin II antagonists (ARb) did not seem to protect against the development of cutaneous melanoma nor was it associated with decreased Breslow's depth. Due to limited statistical precision, however, we cannot exclude an association between ACEi and ARb exposure and either a (moderately) increased or decreased incidence of cutaneous melanoma.

Gender may have a complex relationship with melanoma development and progression as indicated by several findings. Hormonal factors playing a role in these gender differences can still not be excluded. Although not in agreement with previous studies, we showed a modest level of association between (higher) exposure to estrogens, both oral contraceptives (OC) and hormonal replacement therapy (HRT), and increased CM incidence. As CM risk is generally low, we can conclude there is no need to change OC prescription. Abandoning regular prescribing of HRT for uncomplicated menopausal complaints should be advocated, but for more urgent reasons than increased melanoma risk.

Overall, based on the current evidence, one cannot point out which agents are likely to be the most efficacious, but (tertiary) cancer chemoprevention, especially with agents that exhibit additional major health benefits and few (long-term) adverse events, remains an interesting option for patients at high risk of (second) melanomas.

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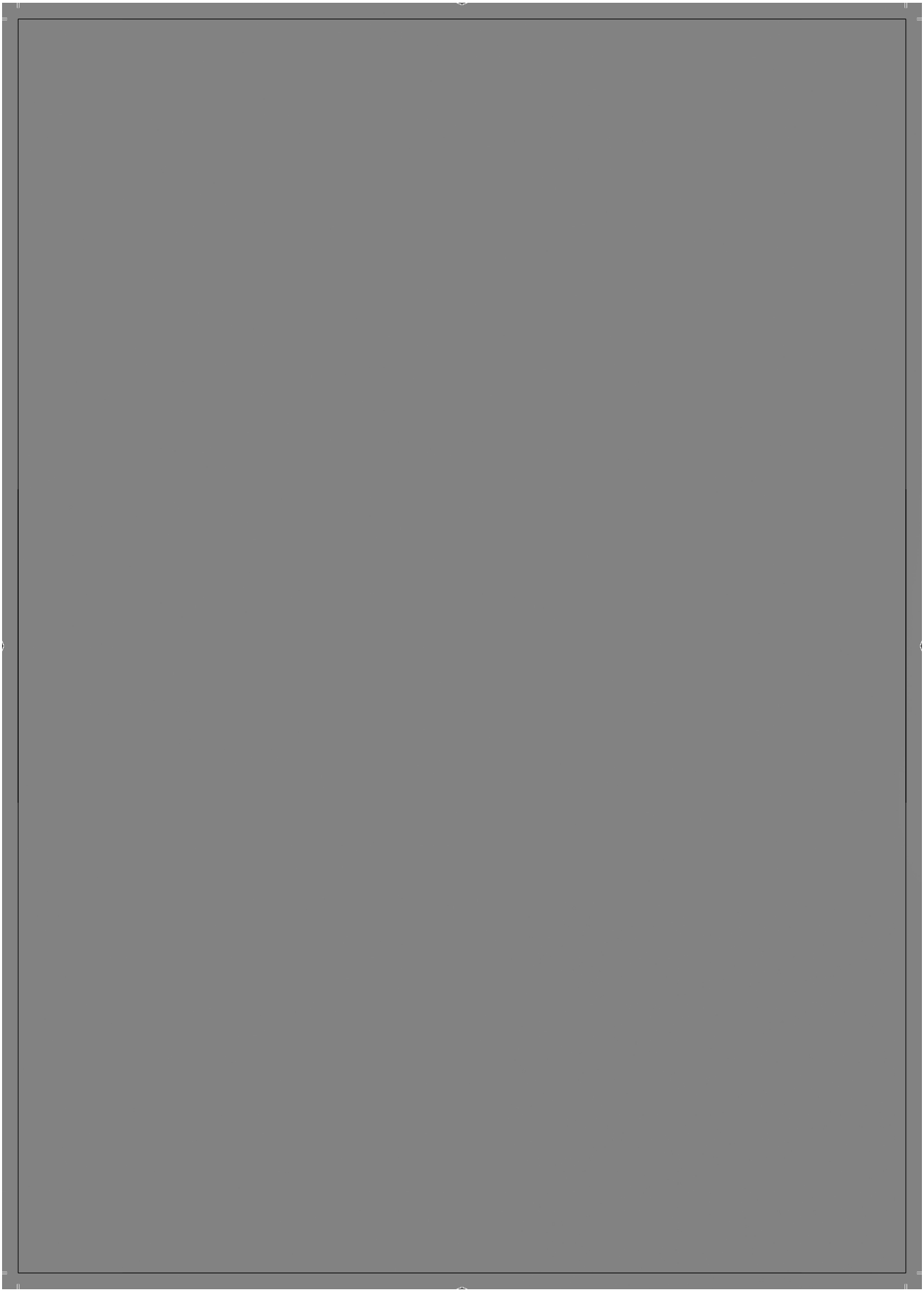

Chapter 11

Summary

Nederlandse samenvatting

Dankwoord

Publicatielijst

Curriculum vitae

Summary

Melanoma is an accumulating health problem in Caucasian populations. Cutaneous melanoma is responsible for the majority of skin cancer deaths and is, therefore, considered the most aggressive form of skin cancer. Moreover, its incidence among Caucasian populations has increased over the past decades whereas mortality rates are stabilizing or in some areas even decreasing. Overall, the total burden of cutaneous melanoma is expected to be increasing in The Netherlands. In rare cases, melanomas arise on noncutaneous sites, so-called extracutaneous melanoma. Due to its rareness, the epidemiology of extracutaneous melanoma is poorly described in literature.

As the burden of cutaneous melanoma is expected to increase, effective treatment options for advanced melanoma are lacking, and beneficial prognosis of melanoma patients strongly depends on early diagnosis, melanoma prevention is likely to be a key issue in melanoma disease control. Although sun protection programs and educational attempts have led to increased awareness, they have not resulted in behavioral changes to sun exposure and protection nor to a decrease in cutaneous melanoma incidence. In addition, most melanoma risk factors are not amenable. Alternative approaches to melanoma prevention, such as cancer chemoprevention are, therefore, important research topics. Several agents, such as statins, non-steroidal anti-inflammatory drugs including aspirin, and angiotensin-converting enzyme inhibitors, have been claimed to have chemopreventive properties. However, it is unknown which of these potential chemopreventive agents have the best potential to be useful and safe.

This thesis presents:

- cancer registry-based studies from The Netherlands on the epidemiology of extracutaneous melanoma and on the burden of disease due to cutaneous melanoma (*epidemiology of melanoma*)
- a qualitative review, based on a systematically literature search, discussing candidate drugs for melanoma chemoprevention, their possible mechanisms of action and summarizing the existing evidence for their chemopreventive efficacy, safety and tolerability (*chemoprevention of melanoma*)
- pharmacoepidemiological studies testing hypotheses on chemopreventive activity on melanoma of statins, non-steroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers (*chemoprevention of melanoma*)
- pharmacoepidemiological studies on the hypothesized association between estrogen use and melanoma (*hormonal and gender differences in melanoma*)

Epidemiology of melanoma

In **chapter 2** we explored the (long-term trends in the) incidence and survival of extracutaneous melanoma in The Netherlands. Based on data from the Netherlands Cancer Registry combined with vital status, we determined the incidence and relative survival of extracutaneous melanoma between 1989 and 2006. We estimated extracutaneous melanoma incidence rates, long-term trends in extracutaneous melanoma incidence, and 5-year relative survival proportions and compared them with those of cutaneous melanoma.

Between 2003 and 2006, extracutaneous melanoma compromised 6.4% of all primary melanomas in The Netherlands. Of all extracutaneous melanoma subsites, ocular melanomas were the most common extracutaneous melanoma subsite with European Standardized incidence Rates of 10.7 and 8.2 per million person-years for males and females, respectively. In addition, ocular melanomas had the best survival. Five-year relative survival for extracutaneous melanoma was worse if compared to cutaneous melanoma for all subsites, but differed largely between anatomical subtypes ranging from 74% for ocular melanomas to 15% for certain subsites of mucosal melanomas. In contrast with cutaneous melanoma for which an annual increase in incidence of 4.4 percent for men and 3.6 percent for women was detected, no statistically significant trends in the incidence of (subsites of) extracutaneous melanoma were observed.

Chapter 3 describes the total burden of cutaneous melanoma within The Netherlands. As we hypothesized, the total burden of cutaneous melanoma increased between 1989 and 2006. The cumulative incidence of cutaneous melanoma almost doubled between 1989 and 2006. In addition, the number of melanoma deaths, the number of years of life lost (YLL), the number of years lost due to disability (YLD), and the number of years of life lived with disease (YLWD) all accumulated in time. On average, patients lived 21.5-28.4 years with a melanoma diagnosis and melanoma resulted in a loss of about 18-20 years per before the age of 95 for those that died of their melanoma. Including all patients diagnosed with an melanoma, not only those that die from it, the average life loss is about 3 years.

Overall, the burden of melanoma to society increased rapidly between 1989 and 2006.

Chemoprevention of melanoma

In **chapter 4** we discuss the available evidence for candidate drugs that have potential to be used in melanoma chemoprevention. Cancer chemoprevention, as defined by Sporn *et al.*, is the use of an agent to reverse, suppress, or prevent premalignant

molecular or histological lesions from progressing to invasive cancer. A systematic literature search was performed and a qualitative review of the selected scientific papers on drug chemoprevention of cutaneous melanoma is presented. This review show that considerable preclinical evidence of efficacy as a melanoma chemopreventive drug exists for aspirin, non-steroidal anti-inflammatory drugs, and statins. However, definite data on efficacy in humans and profound long-term safety data in the required doses are lacking. Therefore, only relatively safe drugs indicated for other health effects but with additional chemopreventive properties in cancer development, such as low-dosed aspirin and statins, can currently be encouraged in people at increased risk of cancer. Less evidence is available for other potential chemopreventive drugs, such as fibrates, retinoids, imiquimod, dehydroepiandrosterone, and acetaminophen.

In the next part of the thesis, we report on a number of pharmacoepidemiological studies using a general population-based dataset (PHARMO) with drug-dispensing data from the pharmaco-morbidity linkage network linked with the national pathological database (PALGA).

Chapter 5 presents a case-control study into the hypothesized association between the incidence and progression of CM and exposure to statins (3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors). We study both the association between use of statins and the incidence of cutaneous melanoma as well as the potential effects of prior statins use on Breslow's thickness at diagnosis of cutaneous melanoma. Cutaneous melanoma risk was not associated with statin use for at least 0.5 year in the 3 years before diagnosis. Although none of the statin-related independent variables consistently supported a risk reduction of statin use on the incidence of cutaneous melanoma, statin use was associated with a reduced Breslow's thickness at diagnosis (–19%, 95% CI = –33, –2.3). This apparent disparity suggests that statins slow melanoma progression but do not protect against cutaneous melanoma development. However, the disparity could also result from the relatively short follow-up.

Surprisingly, after stratification for gender, the finding of a reduced Breslow's thickness among statin users was confirmed for men (–27.8%, 95% CI =–43.7, –7.4) but not for women (–4.8%, 95% CI=–29.6, 28.8). This suggest a clinically relevant reduction of 0.58 mm on average in Breslow's thickness among men. As Breslow's thickness at diagnosis is one of the strongest determinants for prognosis, this should be considered an important finding. However, (residual) bias cannot be excluded and our study is the first study to investigate statin use as a determinant of Breslow's depth. Therefore, validation of these findings is necessary.

In **chapter 6** we study the association between use of non-steroidal anti-inflammatory drugs (NSAIDs) on melanoma development. We included both acetylsaliylic acid (aspirin, ASA) as well as non-ASA NSAIDs.
Cutaneous melanoma incidence was not significantly associated with ever ASA use (adjusted OR = 0.92, 95% CI = 0.76-1.12) or ever non-ASA NSAID use (adjusted OR = 1.10, 95% CI = 0.97-1.24). However, continuous use of low-dose ASAs was associated with a significant reduction of CM risk in women (adjusted OR = 0.54, 95% CI = 0.30-0.99) but not in men (adjusted OR = 1.01, 95% CI = 0.69-1.47). A significant trend (p = 0.04) between categories of ASA use from no use, non-continuous use to continuous use was observed in women.
Continuous use of low-dose ASAs may be associated with a reduced incidence of cutaneous melanoma in women, but not in men.

Angiotensin-converting enzyme inhibitors (ACEi) and possibly angiotensin II receptor blockers (ARb) have also been claimed to have chemopreventive properties. In **chapter 7** we explored the possibility of an association between exposure to ACEi and ARb and the development of cutaneous melanoma.
This study showed no statistically significant associations between the incidence of melanoma and the use of ACEi (adjusted OR= 1.0, 95% CI = 0.8-1.3) or ARb (adjusted OR = 1.0, 95% CI = 0.7-1.5). In addition, the use of ACEi or ARb was not associated with decreased Breslow's thickness. Thus, the use of ACEi or ARb does not seem to protect against the development of cutaneous melanoma. However, due to limited numbers of ACEi and ARb users, we cannot exclude an association between ACEi and ARb exposure and either a (moderately) increased or decreased incidence of cutaneous melanoma. Moreover, residual confounding cannot be excluded. For example, sun exposure may be indirectly related to ACEi and ARb exposure because it may be associated with increased physical activity and a reduced chance of hypertension. Likewise, high social economic status is associated with increased sun exposure and may also be associated with a reduced chance of hypertension. Both these potential biases would in an underestimation of any effect of ACEi and ARb and would thus produce bias toward the null.

Hormonal and gender differences in melanoma

To our surprise, female melanoma cases in the previous studies (chapter 5, 6 and 7) were more likely to be estrogen users than controls. After reviewing literature, we were confronted with several clues indicating possible hormonal influences in melanoma. First, hyperpigmentation is a side-effect of oral contraceptive (OC) use

and may also occur during pregnancy (chloasma) or with the use of hormonal replacement therapy (HRT) both indicating an effect of estrogens on melanocyte proliferation. Second, the pattern of cutaneous melanoma incidence rates with advancing age in women mimic those of breast cancer and differs from the pattern of cutaneous melanoma rates among men. In addition, improved survival among female melanoma patients as compared to males has been demonstrated to remain after adjusting for demographic and tumor characteristics.

As female sex steroids could be involved, we decided to study exposure to estrogens, both oral contraceptives (OC) and hormonal replacement therapy (HRT), and a possible association with the incidence of CM in more detail. In **chapter 8** an association between estrogen use, both OC and HRT, was confirmed for exposure expressed as use for at least half a year, and for the highest categories of cumulative dose and prescription duration. The results suggest a cumulative dose-dependent increased risk of cutaneous melanoma with the use of estrogens, both OC and HRT. However, most previous studies on estrogen use and melanoma are not in agreement with our findings.

As some *in vitro* studies have suggested a direct inhibitory effect on melanoma tumor growth, the use of OC and HRT may be associated with a decreased Breslow's thickness. If so, the clinical impact of an increased cutaneous melanoma incidence with OC and HRT use would be limited. In **chapter 9** we investigated if estrogen use is associated with a decreased Breslow's thickness. However, we could not confirm an association between either OC use or HRT and Breslow's thickness.

In conclusion, the modest level of association between cutaneous melanoma incidence and OC use that resulted from our studies is not in agreement with previous studies, and as cutaneous melanoma risk is generally low, we can conclude there is no need to change OC prescription. Abandoning regular prescribing of HRT for uncomplicated menopausal complaints should be advocated, but for more urgent reasons than increased melanoma risk.

In **chapter 10** the results of this thesis are discussed, possible future directions are outlined and final conclusions are drawn. Since 1989-1998, cutaneous melanoma incidence has further increased between 1998 and 2006 in The Netherlands. Likewise, the total burden of cutaneous melanoma has accumulated over the last decades. These new estimates of the incidence and burden of cutaneous melanoma should be used in health-care planning for melanoma care and surveillance.

Although prognosis is favorable for the majority of melanoma patients, prognosis is poor for some subgroups of patients. Survival for all extracutaneous melanoma

subsites was poor compared to cutaneous melanoma patients but differed substantially for extracutaneous melanoma subsites being most favorable for ocular melanomas.

Several strategies for cancer chemoprevention exist, such as primary, secondary or tertiary cancer prevention and the population or the high risk strategy. As most chemopreventive agents have demonstrated toxicity at some level and long-term compliance is required, the high risk strategy would be the choice of interest for melanoma chemoprevention. Because the absolute risk of getting a melanoma is small, tertiary cancer chemoprevention, at least as a first goal, would seem to be the most realistic as these patients would be at sufficiently high risk of developing a second invasive melanoma. As an additional advantage, in tertiary prevention, one could select a chemopreventive agent for its (additional) potential to prevent metastasis.

Based on current evidence, it is not yet possible to determine which candidate chemopreventive drug(s) is likely to be the most efficacious in medical practice. However, considerable preclinical evidence of chemopreventive efficacy exists for aspirin, NSAIDs, and statins. Lack of definite data on efficacy in humans and profound long-term safety data in the required doses, however, preclude the use of chemopreventive drugs for melanoma in current practice. Future research should explore possible differential effects within a drug class, temporal dose-response relationships, and possible synergistic or antagonistic effects.

In addition, focus should be on how to define high risk subpopulations at whom chemoprevention to target on. Such strategies should be based upon validated prediction models. Candidate items to select for such prediction models could include individual melanoma risk factors including an individual's mutational status of genetic susceptibility genes, validated early biomarkers of invasive melanoma risk, validated molecular and histopathological aspects of any prior tumor, and individual risk factors predicting adverse events of the chemopreventive drug.

In addition, we conclude that gender may have a complex relationship with melanoma development and progression as indicated by several findings. Although these relationships have been studied over a long time, gender differences and possible hormonal influences in melanoma remain are still poorly understood.

To understand these gender differences in melanoma, future studies are warranted. However, as cutaneous melanoma risk is generally low, we can conclude there is no need to change OC prescription. In contrast, abandoning regular prescribing of HRT for uncomplicated menopausal complaints should be advocated, but for more urgent reasons than increased melanoma risk.

Samenvatting

Melanomen vormen een groeiend gezondheidsprobleem in Caucasische populaties. Huidmelanomen zijn verantwoordelijk voor bijna 90% van de huidkankersterfte en zijn daarmee de meest agressieve vorm van huidkanker. Bovendien is de incidentie van huidmelanomen in de laatste decennia sterk toegenomen in Caucasische populaties, terwijl de melanoomgerelateerde mortaliteit stabiliseerde of in sommige gebieden zelfs een geringe afname liet zien. In het algemeen wordt aangenomen dat de totale ziektelast ten gevolge van huidmelanomen in Nederland toeneemt.

In zeldzame gevallen kunnen melanomen op andere lokalisaties dan de huid ontstaan, de zogenaamde extracutane melanomen. Vanwege hun zeldzaamheid is de epidemiologie van dit type melanomen slechts summier beschreven in de medische literatuur.

Aangezien de verwachting is dat de ziektelast ten gevolge van huidmelanomen toeneemt, effectieve behandelopties voor gevorderde stadia van melanomen ontbreken en een goede prognose sterk afhankelijk is van vroege diagnose, wordt preventie van melanomen beschouwd als de sleutel om de ziektelast als gevolg van huidmelanomen in de populatie te beperken.

Hoewel preventieprogramma's gericht op voorlichting en het beperken van (excessieve) blootstelling aan ultraviolette straling hebben geleid tot een toegenomen risicobesef, heeft dit nog niet geleid tot een verandering in zonblootstelling en beschermingsgedrag of tot een afname in de incidentie van huidmelanomen. Bovendien zijn de meeste risicofactoren voor huidmelanomen niet toegankelijk voor preventie. Alternatieve vormen van preventie zoals kanker chemopreventie zijn daarom belangrijke onderzoeksthema's.

Van verschillende geneesmiddelen zoals statines, prostaglandinesynthetaseremmers (NSAIDs) inclusief aspirine en ACE (angiotensin-converting enzyme) remmers worden chemopreventieve eigenschappen verondersteld. Het is echter onbekend welke van deze potentiële chemopreventieve middelen de beste kans maakt om bruikbaar en veilig te zijn.

Dit proefschrift beschrijft:

- Nederlandse kankerregistratie studies naar de epidemiologie van extracutane melanomen en de ziektelast als gevolg van huidmelanomen in de populatie *(epidemiologie van melanomen)*
- een kwalitatief overzicht, gebaseerd op systematisch literatuuronderzoek, waarin de mogelijke werkingsmechanismen van kandidaat geneesmiddelen voor de chemopreventie van melanomen en het huidige bewijs voor hun chemopreven-

tieve effectiviteit, veiligheid en tolerabiliteit wordt bediscussieerd (*chemopreventie van melanomen*)
- farmacoepidemiologische studies waarin hypotheses over de chemopreventieve activiteit van statines, NSAIDs, ACE remmers en angiotensine II antagonisten op huidmelanomen worden getest (*chemopreventiie van melanomen*)
- farmacoepidemiologische studies waarin de veronderstelde associatie tussen gebruik van oestrogenen en melanomen wordt bestudeerd (*hormonale en geslachtsverschillen bij melanomen*)

Epidemiologie van melanomen

In **hoofdstuk 2** bestudeerden we de (lange termijn trends in de) incidentie van extracutane melanomen en de relatieve overleving na vaststellen van de diagnose extracutaan melanoom in Nederland. De incidentie en relatieve overlevingsproportie werd bepaald met behulp van gegevens over de periode 1989-2006 uit de Nederlandse kankerregistratie gecombineerd met informatie over de vitale status. Incidentiecijfers, lange termijn trends in incidentie en 5-jaars overlevingsproporties van extracutane melanomen werden geschat en vergeleken met die van huidmelanomen. Tussen 2003 en 2006 vertegenwoordigden extracutane melanomen 6,4% van alle primaire melanomen in Nederland. Van alle sublokalisaties kwamen oogmelanomen het meeste voor met Europese gestandaardiseerde incidentiecijfers (rates) van 10,7 en 8,2 per miljoen persoonsjaren voor respectievelijk mannen en vrouwen. Bovendien hadden patiënten met oogmelanomen de beste overleving. Vijf-jaars relatieve overlevingsproporties voor extracutane melanomen van alle sublokaties waren slecht vergeleken met huidmelanomen, maar varieerden aanzienlijk tussen de anatomische subtypes van 74% voor oogmelanomen tot 15% voor bepaalde sublokaties van mucosale melanomen. In tegenstelling tot huidmelanomen die een jaarlijkse toename in incidentie van 4,4 procent voor mannen en 3,6 procent voor vrouwen lieten zien, werd geen statistisch significante trend geobserveerd in de incidentie van (subtypen van) extracutane melanomen.

Hoofdstuk 3 beschrijft de totale ziektelast van huidmelanomen in Nederland. Zoals we verwachtten, nam de totale ziekte last als gevolg van huidmelanomen in Nederland toe tussen 1989 en 2006. De cumulatieve incidentie van huidmelanomen verdubbelde bijna tussen 1989 en 2006. Bovendien namen het aantal melanoomgerelateerde sterftegevallen, het aantal verloren levensjaren, het aantal verloren levensjaren als gevolg van invaliditeit en het aantal jaren geleefd met melanoom allemaal toe in de tijd. Gemiddeld genomen, alle melanoompatiënten in acht genomen, blijkt dat een

patiënt met een melanoom ongeveer drie jaar korter leeft dan een vergelijkbaar persoon zonder melanoom. Echter, als alleen patiënten die sterven met als doodsoorzaak een melanoom in ogenschouw worden genomen, dan sterven zij gemiddeld ongeveer 20 jaar eerder in vergelijking met de algemene populatie. Dat betekent daarmee ook dat veel patiënten leven met de diagnose melanoom en met een zekere angst voor progressie van de ziekte en/of het opnieuw ontstaan van huidkanker.

Chemopreventie van melanomen

In **hoofdstuk 4** wordt het beschikbare bewijs voor de verschillende kandidaat geneesmiddelen voor chemopreventie van melanomen bediscussieerd. Kanker chemopreventie, zoals gedefinieerd door Sporn *et al.*, is het gebruik van een middel om te voorkomen dat premaligne laesies progressie vertonen tot een invasieve kanker danwel om deze progressie te onderdrukken of om te keren.
Een systematisch literatuuronderzoek werd uitgevoerd en een kwalitatieve samenvatting van de geselecteerde wetenschappelijke artikelen naar chemopreventie met geneesmiddelen van huidmelanomen wordt gepresenteerd. Dit overzichtsartikel laat zien dat substantieel preklinisch bewijs bestaat voor de effectiviteit van aspirine, NSAIDs en statines als chemopreventief geneesmiddel tegen huidmelanomen. Definitieve gegevens met betrekking tot de effectiviteit in mensen en degelijke gegevens over de lange termijn veiligheid in de benodigde doseringen ontbreken echter. Daarom kunnen momenteel alleen relatief veilige geneesmiddelen die reeds geïndiceerd zijn voor andere indicaties maar tevens aangetoonde chemopreventieve eigenschappen hebben met betrekking tot kankerontwikkeling, zoals laaggedoseerde aspirine en statines, worden geadviseerd voor mensen met een verhoogd risico op kanker. Van andere mogelijke chemopreventieve geneesmiddelen, zoals fibraten, retinoïden, imiquimod, dehydroepiandrosteron en paracetamol, is het bewijs minder overtuigend.

In het volgende deel van dit proefschrift rapporteren we een aantal farmaco-epidemiologische onderzoeken uitgevoerd met behulp van een dataset van het PHARMO instituut voor farmacoepidemiologisch onderzoek met gegevens van afleveringen van geneesmiddelen die gebaseerd is op de algemene populatie en die is gekoppeld aan de nationale pathologie database (PALGA).
Hoofdstuk 5 presenteert een patiënt-controle onderzoek naar de associatie tussen de incidentie en progressie van huidmelanomen en de blootstelling aan statines (3-hydroxy-3-methylglutaryl-coenzyme A reductase remmers). We bestudeerden

zowel de associatie tussen statinegebruik en de incidentie aan huidmelanomen als het mogelijke effect van statinegebruik in het verleden op de Breslow dikte bij diagnose van een huidmelanoom. Het risico op een huidmelanoom was niet geassocieerd met statinegebruik gedurende minimaal een half jaar in de 3 jaar voor diagnose. Hoewel geen van de statinegerelateerde variabelen consequent een verlaagd risico op het onstaan van een melanoom onderschreef, was statinegebruik wel geassocieerd met een verminderde Breslow dikte bij diagnose (–19%, 95% BI = –33, –2.3). Deze ogenschijnlijke tegenstelling suggereert dat statines de progressie van melanomen vertraagt, maar geen effect hebben op het onstaan van een huidmelanoom. Echter, deze discrepantie kan ook het gevolg zijn van de relatief korte follow-up.
Tot onze verrassing, werd onze bevinding van een verminderde Breslow dikte na stratificatie voor geslacht bevestigd voor mannen (–27.8%, 95% BI =–43.7, –7.4), maar niet voor vrouwen (–4.8%, 95% BI=–29.6, 28.8). Dit suggereert een klinisch relevante reductie van gemiddeld 0,58 mm in de Breslow diepte bij mannen. Aangezien Breslow diepte bij diagnose één van de belangrijkste voorspellers voor de prognose is, moet dit als een belangrijke bevinding worden beschouwd. Echter, residuele confounding kan niet worden uitgesloten en ons onderzoek is het eerste waarin statinegebruik als een determinant voor Breslow dikte wordt onderzocht. Daarom is validatie van onze resultaten noodzakelijk.

In **hoofdstuk 6** bestuderen we de associatie tussen het gebruik van prostaglandine-synthetaseremmers (NSAIDs) en het ontstaan van melanomen. We onderzochten zowel acetylsalicylzuur (aspirine, ASA) als de overige NSAIDs. De incidentie van huidmelanomen was niet significant geassocieerd met het ooit gebruiken van aspirine (gecorrigeerde OR = 0.92, 95% BI = 0.76-1.12) of met het ooit gebruiken van één van de overige NSAIDs (gecorrigeerde OR = 1.10, 95% BI = 0.97-1.24). Continu gebruik van een lage dosis aspirine, echter, was bij vrouwen geassocieerd met een significante reductie van het risico op een huidmelanoom (gecorrigeerde OR = 0.54, 95% BI = 0.30-0.99) maar niet bij mannen (gecorrigeerde OR = 1.01, 95% BI = 0.69-1.47). Een significante trend (p = 0.04) tussen de categorieën van aspirine gebruik van geen, niet-continu naar continu gebruik aan aspirine werd gezien bij vrouwen.
Continu laaggedoseerde aspirine kan bij vrouwen, maar niet bij mannen, is geassocieerd met een afgenomen incidentie aan huidmelanomen.

Van angiotensine-converterende enzym inhibitoren (ACE remmers, ACEi) en mogelijk ook angiotensine II receptor blokkers (ARb) is beweerd dat ze chemopreventieve

eigenschappen hebben. In **hoofdstuk 7** exploreren we de mogelijkheid van een associatie tussen blootstelling aan ACEi en ARb en de ontwikkeling van huidmelanomen. Dit onderzoek liet geen statistisch significante associatie zien tussen de incidentie van huidmelanomen en het gebruik van ACEi (gecorrigeerde OR= 1.0, 95% BI = 0.8-1.3) of ARb (gecorrigeerde OR = 1.0, 95% BI = 0.7-1.5). Bovendien was het gebruik van ACEi of ARb niet geassocieerd met een verlaagde Breslow dikte. Het gebruik van ACEi en ARb lijkt dus geen beschermend effect te hebben op de ontwikkeling van huidmelanomen. Als gevolg van de lage aantallen ACEi en ARb gebruikers kunnen we echter een associatie tussen blootstelling aan ACEi en ARb met een (matig) verhoogd of verlaagde incidentie aan huidmelanomen niet geheel uitsluiten. Bovendien kan residuele confounding niet uitgesloten worden. Verhoogde blootstelling aan zonlicht bijvoorbeeld, kan indirect gerelateerd zijn blootstelling aan ACEi of ARb, omdat toegenomen fysieke activiteit geassocieerd is met een lagere kans op hypertensie (een indicatie voor het voorschrijven van ACEi of ARb). Op vergelijkbare wijze kan een hogere sociaal economische status geassocieerd zijn met zowel een verhoogde zon-blootstelling als een verminderde kans op hypertensie. Allebei deze mogelijke bronnen van vertekening zouden leiden tot een onderschatting van een effect van ACEi en ARb op de incidentie van huidmelanomen en zouden dus een bias geven richting nul(hypothese).

Hormonale en geslachtsverschillen bij melanomen

Tot onze verrassing waren vrouwelijke melanoompatiënten in de voornoemde studies (hoofdstukken 5, 6 en 7) vaker gebruikers van oestrogenen dan hun controles. Nader literatuuronderzoek toonde verschillende aanwijzingen die hormonale invloeden op melanomen suggereren. Allereerst, is hyperpigmentatie een bijwerking van orale anticonceptiva (OAC) en treedt ook wel op tijdens de zwangerschap (chloasma) of met het gebruik van hormonale suppletie therapie (HST) wat een effect van oestrogenen op de melanocyten proliferatie suggereert. Ten tweede is het patroon van de incidentie van huidmelanomen bij vrouwen van toenemende leeftijd vergelijkbaar met dat van borstkanker en verschilt dit incidentiepatroon met dat van mannen. Bovendien hebben vrouwelijke melanoompatiënten een betere overleving vergeleken met mannen die ook na correctie voor verschillen in demografische en tumor karakteristieken blijft bestaan.

Aangezien vrouwelijke hormonen een rol zouden kunnen spelen, besloten we een meer gedetailleerde analyse uit te voeren naar een mogelijke associatie tussen gebruik van oestrogenen, zowel orale anticonceptiva (OAC) als hormonale suppletie-therapie (HST), en huidmelanomen.

In **hoofdstuk 8** werd een associatie tussen oestrogeengebruik en de incidentie van huidmelanomen bevestigd voor zowel OAC als HST en voor het totaal aan oestrogeengebruik uitgedrukt als minstens een half jaar gebruik, de hoogste categorie van de cumulatieve dosering en de prescriptieduur. De resultaten suggereren een cumulatief dosisafhankelijk verhoogd risico op huidmelanomen bij oestrogeengebruik (zowel OAC als HST). De meeste voorgaande onderzoeken naar oestrogeengebruik en melanomen komen echter niet overeen met onze resultaten.
Aangezien sommige *in vitro* studies een direct inhiberend effect van oestrogenen op de celgroei van melanomen suggereren, kan gebruik van OAC en HST gerelateerd zijn aan een verlaagde Breslow dikte. Als dat zo is, dan beperkt dat de eventuele klinische impact van een toegenomen incidentie aan huidmelanomen bij OAC en HST gebruik.
In **hoofdstuk 9** onderzochten we of oestrogeengebruik geassocieerd is met een afgenomen Breslow dikte. Een associatie tussen ofwel OAC gebruik ofwel HST gebruik en Breslow dikte bij diagnose konden we echter niet bevestigen.
Kortom, de matige associatie tussen de incidentie van huidmelanomen en OAC gebruik die wij in onze onderzoeken aantoonden komt niet overeen met eerdere studies en aangezien het risico op een huidmelanoom gering is kunnen we concluderen dat dit geen aanleiding vormt om het voorschrijfgedrag voor OAC te wijzigen. Zeer terughoudend zijn met het voorschrijven van HST voor ongecompliceerde menopauzale klachten is te adviseren, maar vanwege andere redenen dan een verhoogd risico op huidmelanomen.

In **hoofdstuk 10** worden naast de resultaten uit dit proefschrift, mogelijke toekomstige richtingen voor onderzoek en de uiteindelijke conclusies besproken. Sinds 1989-1998 is de incidentie van huidmelanomen verder toegenomen in Nederland. Evenzo is de totale ziektelast van huidmelanomen toegenomen in de laatste decennia. Deze nieuwe schattingen van de incidentie en ziektelast van huidmelanomen zou moeten worden gebruikt bij de (capaciteits)planning van de behandeling en het vervolgen van patiënten met een melanoom voor de toekomst.
Hoewel de prognose voor de meerderheid van melanoompatiënten gunstig is, is die voor sommige subgroepen van patiënten slecht. De overlevingsproportie voor alle sublokaties van extracutane melanomen was slecht vergeleken met die van huidmelanomen en varieerde aanzienlijk voor de verschillende sublokaties met de meest gunstige overleving voor oogmelanomen.
Er bestaan verschillende strategieën voor kanker chemopreventie, zoals primaire, secundaire of tertiaire kanker preventie en de populatie of hoogrisico strategie. Aangezien de meeste chemopreventieve middelen (enige vorm van) toxiciteit hebben

getoond en lange termijn therapietrouw is vereist, lijkt de hoogrisico strategie aangewezen voor de chemopreventie van melanomen. Omdat het absolute risico voor het krijgen van een melanoom klein is, lijkt tertiaire chemopreventie, in eerder geval als eerste doel, het meest realistisch aangezien voor deze patiënten een voldoende hoog risico geldt op een tweede melanoom. Bijkomend voordeel bij tertiaire preventie is dat een chemopreventief middel gekozen kan worden dat potentie heeft om metastasering te voorkomen.

Op basis van het huidige bewijs in de wetenschappelijke literatuur is het nog niet mogelijk om aan te geven welk chemopreventief geneesmiddel hoogst waarschijnlijk het meest effectief zal zijn. Voor aspirine, NSAIDs en statines bestaat echter substantieel preklinisch bewijs. Gebrek aan gegevens met betrekking tot effectiviteit in mensen en lange termijn veiligheid in de benodigde doseringen sluiten het gebruik van chemopreventieve geneesmiddelen in de melanomen patiëntenzorg op dit moment uit. Toekomstig onderzoek zou zich kunnen richten op mogelijke differentiële effecten binnen een geneesmiddelklasse, dosisrespons relaties in de tijd en mogelijke synergistische of antagonistische effecten.

Daarnaast zou de aandacht in nieuw onderzoek moeten uitgaan naar hoe hoogrisico subpopulaties waarop chemopreventie zich zou moeten richten het beste gedefinieerd kunnen worden. Dergelijke strategieën zouden gebaseerd moeten zijn op gevalideerde predictiemodellen. Kanididaat variabelen om te selecteren voor deze predictie-modellen zouden individuele risicofactoren voor melanomen kunnen zijn, zoals de mutatiestatus van ziektegerelateerde genen, gevalideerde vroege biomarkers van invasieve melanomen, gevalideerde moleculaire en histopathologische aspecten van eerdere tumoren en individuele risicofactoren die bijwerkingen en complicaties als gevolg van het chemopreventieve geneesmiddel voorspellen.

We concluderen bovendien dat geslacht een complexe relatie met het ontstaan en de progressie van melanomen heeft. Hoewel deze relaties al over een lange periode worden bestudeerd, zijn geslachtsverschillen en mogelijke hormonale invloeden nog steeds slecht begrepen fenomenen.

Om deze geslachtsverschillen bij melanomen beter te begrijpen is toekomstig onderzoek vereist. Aangezien het risico op een melanoom klein is, kunnen we echter concluderen dat er geen reden is het voorschrijfgedrag voor OAC te veranderen. Het voorschrijven van HST voor ongecompliceerde menopauzale klachten, echter, moet wel afgeraden worden, maar vanwege meer dringende redenen dan een verhoogd risico op een melanoom.

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Curriculum Vitae

Elsje (Els) Rosalie Koomen is geboren op 20 december 1976 in Nijmegen. Na het behalen van haar VWO diploma in 1995 begon zij met de studie Farmacie aan de Universiteit Utrecht. Tijdens de doctoraalfase heeft zij, onder begeleiding van Prof. A.C.G. Egberts, farmaco-epidemiologisch onderzoek verricht naar verschillende aspecten van de antibiotica gentamicine, tobramycine en vancomycine in twee Nederlandse ziekenhuizen. Dit onderzoek omvatte zowel studies naar *therapeutic drug monitoring*, risicofactoren voor hoge dalspiegels, risicofactoren voor aminoglycoside- gerelateerde nefrotoxiciteit en microbiologische testuitslagen bij gebruikers van genoemde antibiotica. Tijdens de studie was zij lid van theatergroep Instant, lid van de Onderwijs Evaluatie Redactie van de studie Farmacie, en medeoprichtster en penningmeester van theatergroep 'Stichting Roerend Goed' in Utrecht.

In 2001 behaalde zij haar apothekersdiploma, waarna ze enkele maanden als projectapotheker heeft gewerkt in het Ruwaard van Putten ziekenhuis en vervolgens in de ziekenhuisapotheek van het Groene Hart ziekenhuis te Gouda. Eind 2003 startte ze met de opleiding tot ziekenhuisapotheker in het Leids Universitair Medisch Centrum (LUMC, opleider: Prof. Dr. H.-J. Guchelaar). Tijdens haar opleiding voerde ze, in een samenwerkingsverband van de afdeling Klinische Farmacie en Toxicologie met het PHARMO instituut en stichting PALGA, een farmaco-epidemiologisch onderzoek uit naar de effecten van statines op huidmelanomen onder begeleiding van onder andere Dr. T. Nijsten en Prof. Dr. H.-J. Guchelaar. Hiervoor ontving zij in 2007 de Sanofi Aventis Award.

Na haar registratie als ziekenhuisapotheker medio 2007, combineerde ze het werk als ziekenhuisapotheker in de farmaceutische dienstverlening in het LUMC met het promotieonderzoek en de Masteropleiding Epidemiologie aan het EMGO instituut van de Vrije Universiteit van Amsterdam. Deze opleiding heeft ze in maart 2009 onder begeleiding van Prof. Dr. J. Vandenbroucke en Prof. Dr. J.W.W. Coebergh afgerond (cum laude). Inmiddels is ze begonnen in haar nieuwe functie van Qualified Person en hoofd Quality Assurance van de ziekenhuisapotheek in het LUMC.