Use of prescribed drugs and risk of cutaneous melanoma

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"The scientific man does not aim at an immediate result. His work is like that of the planter – for the future. His duty is to lay the foundation for those who are to come, and point the way. He lives and labours and hopes."

- Nikola Tesla

Sammendrag (Norwegian)

Blant hudkreft, er melanom proporsjonalt i mindretall, men forårsaker høyest antall dødsfall. I Norge er melanom den kreftformen med størst økning i forekomst og representerer en økende trussel mot folkehelsen. Den primære risikofaktoren for utvikling av melanom er først og fremst eksponering for ultrafiolett (UV) stråling fra sol (og solarium). Utvikling av melanom kan også avhenge av flere andre faktorer knyttet til biologiske karakteristikker og livsstil. Dette kan også inkludere bruk av enkelte reseptbelagte legemidler. Flere legemiddelgrupper kan skape immunmodulerende og/eller fotosensitiviserende effekter. Mange av disse brukes over lang tid og kan påvirke risikoen for melanom. Mer kunnskap om rollen til disse potensielle risikofaktorene kan bidra til å motvirke den stadig økende melanomforekomsten i flere land, inkludert Norge.

Denne avhandlingen undersøkte sammenhengen mellom bruk av reseptbelagte antidepressiva, immunmodulerende og antihypertensive legemidler, og risikoen for melanom i Norge ved bruk av nasjonale helseregistre. Studien har et kasus-kontroll design. Informasjon om alle første primære tilfeller av melanom i hud diagnostisert i perioden 2007–2015 (kasus) ble innhentet fra Kreftregisteret. Kontroller med likt kjønn og fødselsår (1:10) ble identifisert i Folkeregisteret, med bruk av risk-sett utvelgelse. Informasjon om reseptbelagte legemidler i 2004–2015 ble innhentet for kasus og kontroller fra Reseptregisteret. Informasjon om paritet ble hentet fra Medisinsk fødselsregister (for kvinner). Vi estimerte rate ratio for hver legemiddelgruppe basert på antall resepter og kumulativ dose, justert for annen legemiddelbruk og bostedsregion, som proxy for UV eksponering. For hver av legemiddelgruppene ble det også estimert rate ratio for histologisk type, tumor lokalisasjon, klinisk stadium, kjønn, alder ved diagnose/indeksdato og bostedsregion.

Bruk av antidepressiva var assosiert med redusert risiko for melanom, inkludert selektive serotoninreopptakshemmere og blandet bruk av antidepressiva. Bruk av immunsuppressive legemidler var derimot assosiert med økt risiko for melanom, inkludert metotreksat, men spesielt legemidler brukt etter organtransplantasjon. Bruk av antihypertensiva var også assosiert med økt melanomrisiko. Dette gjaldt for bruk av diuretika, kalsiumkanalblokkere og legemidler for renin-angiotensinsystemet. Ingen lineær dose-respons ble funnet for noen av legemiddeltypene, basert på kumulativ definert daglig dose. Funnene i denne avhandlingen indikerer at brukere av antidepressiva har redusert melanomrisiko, men at dette også kan forklares av redusert UV-eksponering blant brukere. Brukere av de fleste antihypertensive og spesielt immunsuppressive legemidler har økt melanomrisiko. Både modulering av immunmekanismer og fotosensitivisering kan ha bidratt til dette. For bedre forebygging av melanom kunne de relevante pasientgruppene i tillegg til regelmessige hudkontroller, gjøres oppmerksomme på viktigheten av forsiktig soleksponering.

Summary

Cutaneous melanoma is the deadliest form of skin cancer in terms of number of mortalities and is the most rapidly growing malignancy in Norway, becoming a considerable public health threat. Exposure to ultraviolet radiation (UVR) from the sun (and solarium) is recognized as the primary risk factor for the development of melanoma. Melanoma development however, can depend on a variety of other biological host factors and lifestyle factors. This may include prescription drug use which has grown in parallel with melanoma incidence rates. Several major types of prescription drugs induce immunomodulating and/or photosensitizing effects which could influence the risk of melanoma, including antidepressant, immunosuppressive and cardiovascular drugs. Many are also used long-term and have been associated with increased skin cancer risk, including melanoma. Increased knowledge of this potential risk factor is important as it could help mitigate the increasingly severe public health impact caused by a rising number of melanoma cases in several countries, including Norway.

This thesis investigated the associations between the use of antidepressant, immunomodulating and antihypertensive prescription drug groups, and melanoma risk in Norway during the period 2004–2015 using nationwide population-based health registers. Data regarding all diagnosed first primary cutaneous melanoma cases in 2007–2015 was sourced from the Cancer Registry of Norway and matched by sex and age of birth to population controls (1:10) from the Norwegian National Registry using risk set sampling. Information on prescribed drugs from 2004–2015 was obtained for cases and controls by linkage to the Norwegian Prescription Database. Data on parity was sourced from the Medical Birth Registry of Norway (for women). Using a nested case-control design, we estimated rate ratios for each drug group by number of prescriptions and cumulative dose, adjusted for other drug use and region of residency, as a proxy for ambient UVR exposure. Furthermore, this thesis investigated the melanoma risk for each such drug group stratified by histological subtype, body site, clinical stage, sex, age at diagnosis/index date and ambient UVR exposure.

Use of antidepressant drugs was associated with a decreased risk of melanoma, including selective serotonin reuptake inhibitors and mixed use of antidepressant drugs. Use of immunosuppressant drugs was associated with an increased risk of melanoma, including methotrexate, but particularly drugs prescribed to organ transplant recipients. Use of antihypertensives was associated with an increased risk of melanoma for users of diuretics, calcium channel blockers and agents of the renin angiotensin system, with similar results when using an active comparator design. No linear dose response relationship was found for any of the drug types, based on cumulative defined daily dose. The findings of this thesis suggest that while antidepressants may induce cancer-inhibiting effects, less UVR exposure among users may be an equally plausible explanation. Findings also indicate that use of certain antihypertensive, and particularly immunosuppressant drugs increases melanoma risk, with drug-induced photosensitization being a likely contributor. These patient groups could in addition to regular skin check-ups, pursue a more cautious approach to sun exposure.

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Scientific Papers

Paper 0

- Berge LAM, Andreassen BK, Stenehjem JS, Larsen IK, Furu K, Juzeniene A, Roscher I, Heir T, Green A, Veierød MB, Robsahm TE. Cardiovascular, antidepressant and immunosuppressive drug use in relation to risk of cutaneous melanoma: a protocol for a prospective case-control study. BMJ Open 2019; 9(2): e025246.

Paper I

- Berge LAM, Andreassen BK, Stenehjem JS, Heir T, Furu K, Juzeniene A, Roscher I, Larsen IK, Green AC, Veierød MB, Robsahm TE. Use of antidepressants and risk of cutaneous melanoma: A prospective registry-based case-control study. Clin Epidemiol 2020;12: 193-202.

Paper II

- Berge LAM, Andreassen BK, Stenehjem JS, Heir T, Karlstad Ø, Juzeniene A, Ghiasvand R, Larsen IK, Green AC, Veierød MB, Robsahm TE. Use of immunomodulating drugs and risk of cutaneous melanoma: A nationwide nested case-control study. Clin Epidemiol 2020;12: 1389-1401.

Paper III

- Ghiasvand R, Berge LAM, Andreassen BK, Stenehjem JS, Heir T, Karlstad Ø, Juzeniene A, Larsen IK, Green AC, Veierød MB, Robsahm TE. Use of antihypertensive drugs and risk of cutaneous melanoma: A nationwide nested case-control study (Manuscript ready for submission).

Abbreviations

All are defined in full at their first instance in the text:	
ACEi:	Angiotensin-converting-enzyme inhibitors
ARB:	Angiotensin II receptor blockers
ATC:	Anatomical therapeutic chemical
BCC:	Basal cell carcinoma
CI:	Confidence interval
CRN:	Cancer registry of Norway
DDD:	Defined daily doses
EU-ADR:	European medicines agency database of suspected adverse drug reactions
IARC:	International agency for research on cancer
ICD-O-3:	International classification of diseases of oncology 3rd edition
ICD-10:	International classification of diseases 10th revision
KC:	Keratinocyte cancer
MAOI:	Monoamine oxidase inhibitor
MAPK:	Mitogen-activated protein kinase
MBRN:	Medical birth registry of Norway
NM:	Nodular melanoma
NorPD:	Norwegian prescription database
NOWAC:	Norwegian women and cancer
NNR:	Norwegian national registry
OTR:	Organ transplant recipient
PIN:	Personal identification number
RAS:	Renin angiotensin system
RCT:	Randomized control trial
ROS:	Reactive oxygen species
RR:	Rate ratio
SCC:	Squamous cell carcinoma
SPF:	Sun protection factor
SSM:	Superficial spreading melanoma
SSRI:	Selective serotonin reuptake inhibitor
TCA:	Tricyclic antidepressant
US:	United States of America
UVA, -B, -C:	Ultraviolet A, -B, -C
UVR:	Ultraviolet radiation
WHO:	World Health Organization

Table of Contents

1. Introduction

1.1 Cutaneous melanoma

Skin cancer is comprised of a spectrum of cutaneous complications, all characterized by a malignant transformation of one or more types of skin cells. Keratinocyte cancer (KC) includes a series of non-melanoma skin cancer types that affect keratinocytes. KC is mainly used to describe basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), but can also include rare neoplasms like cutaneous lymphomas, adnexal tumors, sarcomas and Merkel-cell carcinomas.[1] The incidence rate (per 100,000, Nordic standard) of cutaneous melanoma (hereafter melanoma), is lower than KC (37.5 and 49.6, respectively), yet its mortality rate is far higher than KC (5.8 and 1.1, respectively).[2] This makes it the deadliest type of skin cancer in terms of number of deaths. Melanoma has been identified as a separate disease in humans as far back as 1812, with the publication of the article "The Melanoses", by Rene Laennec. The condition is characterized by a malignant transformation of melanocytes, leading to neoplasms with a variety of clinical and histopathological classifications, depending on genetic factors and the tissue types in which it arises.[3]

Melanomas arising from melanocytes in epithelial tissues are classified into 4 groups of subtypes (Figure 1a). The primary group includes superficial spreading melanomas (SSM), nodular melanomas (NM) and spitzoid melanomas. These may also include non-malignant variants, such as dysplastic and acquired nevi, Spitz nevi and atypical Spitz tumors. Other groups include lentigo and desmoplastic melanomas, which typically arise on the head and neck[4], as well as mucosal melanomas, most often arising on the genital areas and throat. Finally, there is the acral melanoma type, which arises on nails, palms and soles (Figure 1a).

Of the most prolific melanoma clinical subtypes, SSM and NM make up the two most frequent forms, followed by lentigo and acral melanomas. SSM is characterized by a distinct radial growth phase and represents approximately two thirds of all melanomas in fair-skinned populations (60–70%). NM makes up 15-20% of all melanomas in fair-skinned populations and is characterized by a distinct vertical growth phase.[5] Melanomas that arise in areas outside the epithelium are rarer and can develop in the eye as uveal melanomas and in internal organs as visceral melanomas. Non-epithelial melanomas may also arise in dermal tissue as blue nevus-like melanoma and melanomas of congenital nevus (Figure 1b).[4] The focus of this this thesis however, is restricted to epithelial melanoma of the skin (melanoma).
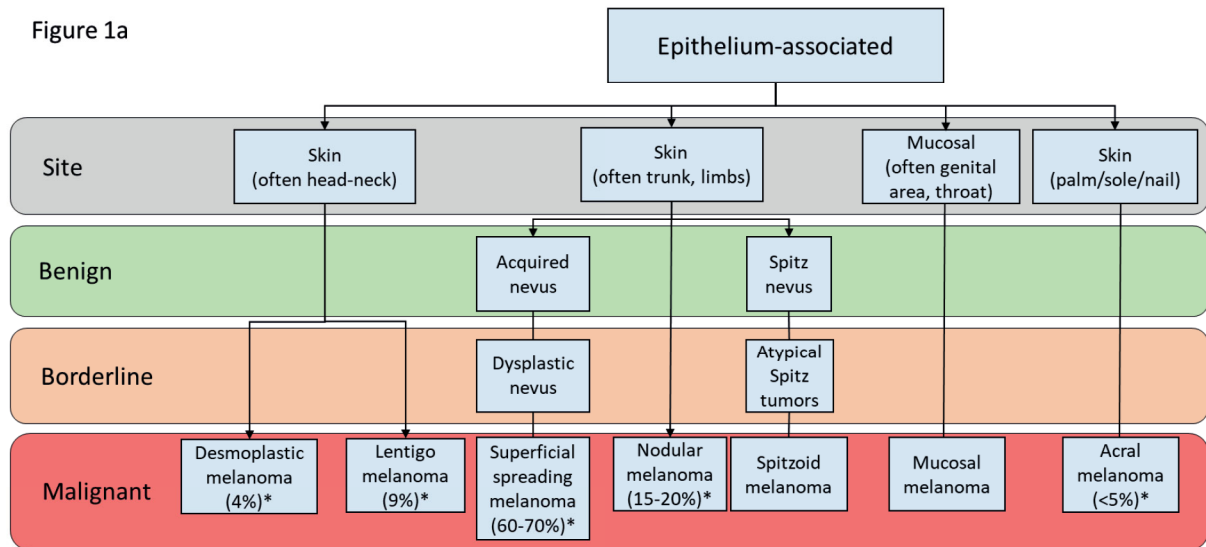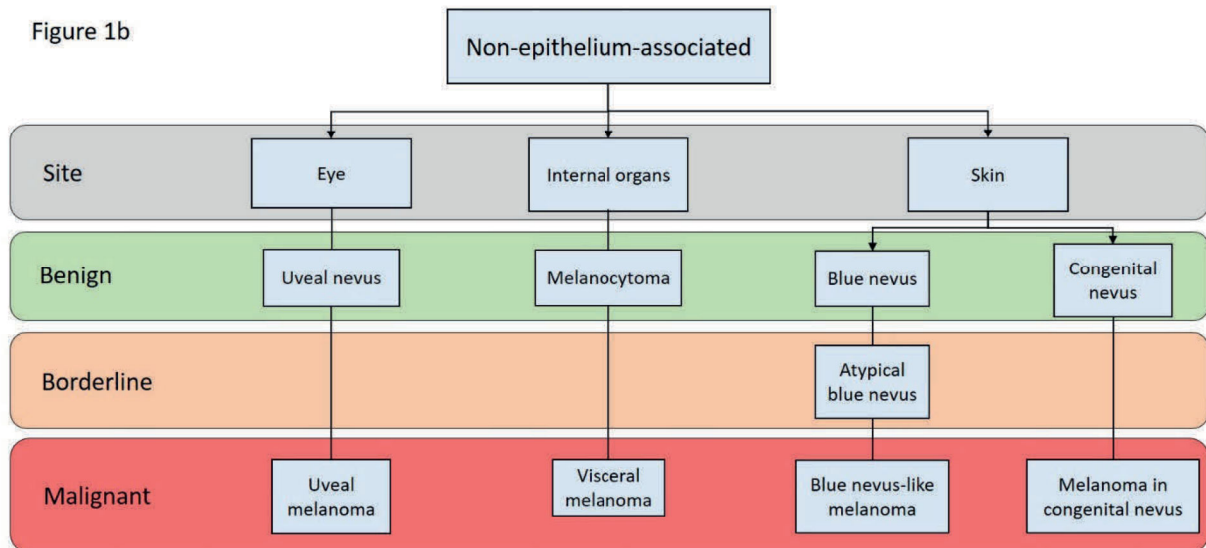
Figure 1. Clinical groupings of melanoma based on site, with degrees of malignancy. **1a:** Melanomas arising from melanocytes associated with the epithelium. **1b:** Melanomas arising from melanocytes not associated with the epithelium. Adapted from Bastian, 2014[6] and Shannan et al. 2016.[4] ***** Percentage is based on all cases of melanoma, but do not add up to 100% due to other and unclassified melanoma cases.

As well as these distinct subtypes, melanomas also feature a wide spectrum of phenotypic and genetic variation. This is prompting genetic analyses for the purposes of making more informed clinical decisions in melanoma diagnosis and therapy.[5] Moreover, melanoma is the cancer form in humans that carries the most mutations, leading to a wide variety of genetic variations. This affects critical pathological factors, like cell growth and migration, as well as the expressed tumor-associated antigens. [4,7,8] This genetic heterogeneity not only changes over time, but often varies within the same tumor mass. Melanomas are inherently dependent on the mitogen-activated protein kinase (MAPK) pathway, both for melanocyte growth and migration.[7] Constitutive activation of the MAPK pathway is for example, often due to activating mutations in the BRAF (50% of melanomas) and NRAS (20-25% of mutations) genes.[4] Several other genes, such as the microphthalmia-associated transcription factor are critical for regulating melanocyte development. The genetic alterations for melanoma also include several other gain and loss-of-function mutations.[4] However, while

important from a clinical perspective, describing the entirety of this intertumoral genetic heterogeneity is considered to be outside the scope of this thesis.

1.2 Melanoma epidemiology

For the past decades, the incidence and mortality due to melanoma have been increasing rapidly worldwide, particularly among fair-skinned populations.[9,10] However, while melanoma rates in northern European countries and in fair-skinned North American populations continue to rise, this increase has stabilized in Oceania.[11] In Norway, melanoma was rare in the early 1950s, when the Cancer Registry of Norway (CRN) began registering cancer data, but has with increasing speed become among the most frequently diagnosed cancer types, particularly so during the last two decades. Based on the Norwegian standard population, the age-standardized incidence rates of melanoma per 100,000 person-years have from 2009 to 2018 increased from 32.1 to 44.8 (39.6%) in males, and 29.5 to 40.9 (38.6%) in females. This makes melanoma the most rapidly growing cancer type for both sexes in Norway today (Figure 2).[12]

Cutaneous melanoma 1955-2019
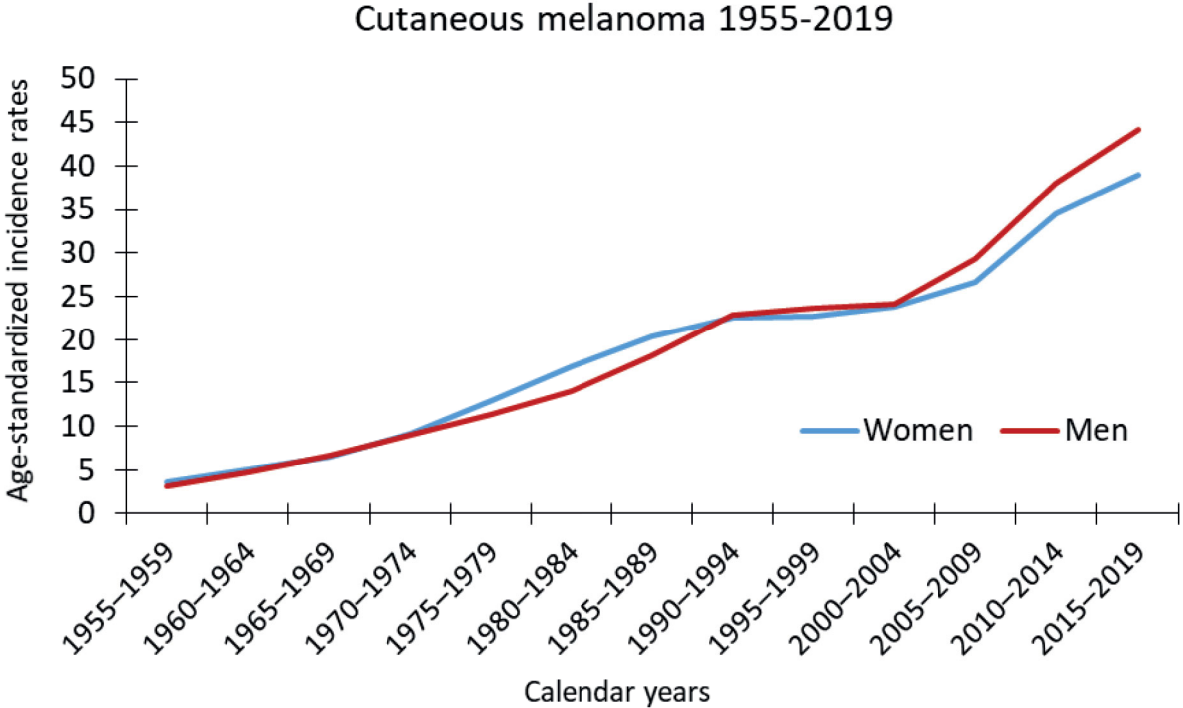
Figure 2: Age standardized (Norwegian Standard) incidence rate (per 100,000) of melanoma in Norway in men and women in the period 1953–2019.[12]

The age-specific incidence rates of the last two decades show that the steepest increase has occurred among men in the oldest age group (Figure 3). The incidence of melanoma increases with age, and the median age at diagnosis has increased from 56 years in 1985–89 to 66 years in 2014–19.[12] However, melanoma is also one of the most common malignancies among young adults worldwide, particularly among

females.[9,12,13] By 2019 in Norway, melanoma of the skin was the second most common cancer in females aged 15–24, accounting for 13.3% of cancer diagnoses in this age group.[12] In males at the same age, melanoma accounted for 4.5% of the cancer diagnoses. Melanoma was also the second most common cancer in both sexes in the age group 25–49.[12] Norway ranks among the top five countries worldwide, in terms of both melanoma incidence (5th) and mortality (2nd).[14] In recent years, the increasing incidence has been accompanied by a flattening or decreasing mortality in comparable countries, while the rate in Norway has continued to increase.[15] In 2018, 307 deaths and an age-standardized (Norwegian standard population) mortality rate of 7.6 and 3.8 per 100,000 person-years was registered for males and females, respectively.[12]
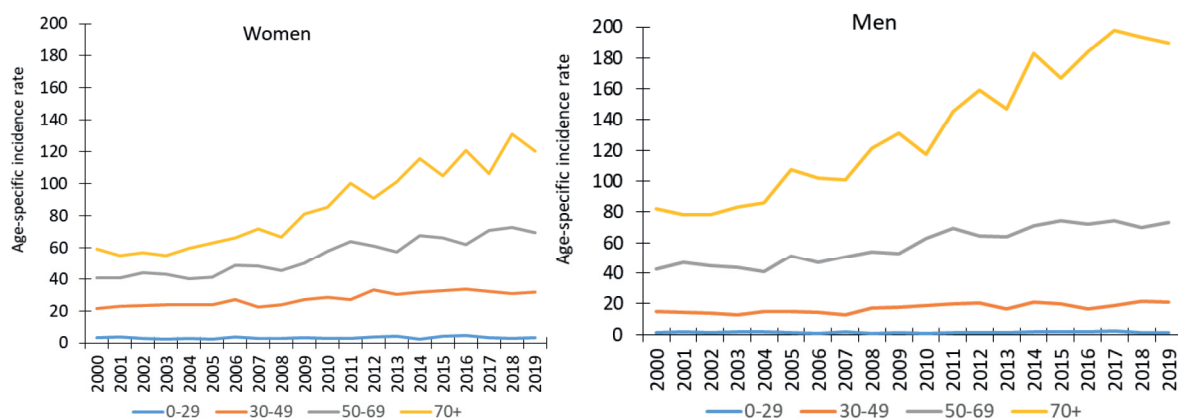
Figure 3: Age-specific incidence rates (per 100,000) of melanoma in Norway among women and men for the period 2000–2019, for the age groups 0-29, 30-49, 50-69 and 70+.

When diagnosed in a local stage, melanoma has high survival rates, and as most cases are diagnosed without metastasis in Norway, the five year relative survival is high, being 88.6% in men and 94.0% in women.[12] Implementation of new medical treatments for advanced stage melanoma in 2012-13 is suggested to be the main contribution to the most recent increase in survival from 12% to 34% in men and 30% to 50% in women with advanced stage disease.[12]

1.3 Risk factors

1.3.1 Ultraviolet radiation (UVR) exposure

It is well-established that the major risk factor for melanoma overall is ultraviolet radiation (UVR) exposure, with the sun being the main source. The entire UVR spectrum is in fact deemed carcinogenic to humans.[16] Of all the UVR that reaches the surface of the earth, 95% is UVA (315-400 nm) and the remaining 5% UVB (280-315 nm), as all the UVC (100-280 nm) and most UVB is removed by its passage through the upper atmosphere. UVB is primarily regarded as a risk factor for sunburns, while both UVA and UVB are suggested to increase the risk of melanoma.[13,16] The melanin of melanocytes serves both to absorb harmful UVR and neutralize free-radicals generated from this exposure. The same exposure however, can induce the malignant transformations leading to melanoma.

It is estimated that more than 75% of all global melanoma cases can be attributed to excess UVR exposure, both from the sun and artificial sources.[17] In Oceania, excess UVR exposure is estimated to account for 96% of all melanomas.[17] A similar proportion (95%) has been estimated for the Nordic countries, despite their higher latitude and different climate.[18] The ambient UVR received is dependent on region of residence, with UVR intensity increasing with proximity towards the equator.[9,13,19] Within Norway, the climate and latitudes create large contrasts in ambient UVR doses.[20] The impact of this ambient UVR exposure is reflected in the geographic distribution of melanoma as well as in migration studies [21,22], including within Norway.[23] However, regional ambient UVR exposure is not the only factor associated with melanoma rates. Increasing travel to destinations with high sun-exposure is theorized to be associated with an increased melanoma incidence in areas with relatively low UVR doses, reducing the geographical contrasts between regions with different ambient UVR exposure levels (Figure 4).[24]
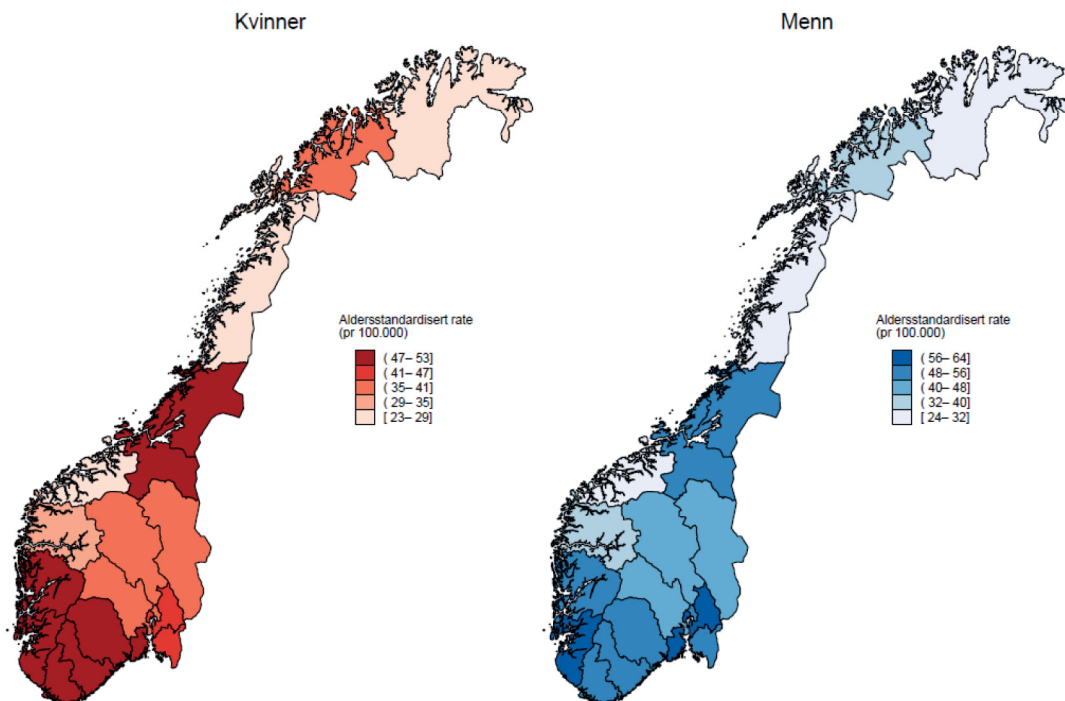
Figure 4: The age-standardized (Norwegian Standard) incidence rates of melanoma per 100,000 for women (Kvinner) and men (Menn) in Norway in 2019.[12]

UVR exposure is typically classified into one of four categories: recreational (intermittent, intense exposure associated with behaviors such as sunbathing), ambient (associated with place of living), everyday exposure (continuous, often low-intensity chronic exposure typical of outdoor occupations) and sunburn (a marker of severe acute UVB exposure).[13] Intermittent UVR exposure is associated with an increased risk of melanoma, with an accumulative effect. The association with melanoma risk is non-existent or even weakly inverse for chronic exposure. Meanwhile, total sun exposure shows a positive, yet weaker association with melanoma than intermittent/short UVR exposure alone.[25] Explanations for these relationships propose that chronic and low intensity UVR exposure promotes gradual epithelial thickening that with a concurrent tanning effect, may ensure a degree of protection against subsequent UVR exposure.[25,26]

The intensity and length of UVR exposure may lead to various manifestations of melanoma, both in terms of phenotype and body site (Figure 1a). Melanomas may develop at different body sites due to certain sun exposure patterns and can thus be divided into chronically and non-chronically sun damaged melanomas (the divergent pathways hypothesis). Chronically sun damaged melanomas typically originate on the head/neck of older individuals (>55 years of age) with few nevi (nevi indicate susceptibility to melanocytic proliferation), while non-chronically sun damaged melanomas typically affect the more intermittently sun-exposed areas such as the trunk and extremities of younger individuals (<55 years of age) with more nevi.[27-29] Intermittent UVR exposure early in life has also been associated with a subsequently higher risk of melanoma later in life [30], suggesting that the period of childhood and adolescence is of particular importance. However, a nation-wide study from Norway, demonstrated that the area of residence both before and after the age of 17 affected melanoma risk, indicating that UVR exposure at any age is important when considering lifetime risk of melanoma.[23]

Other UVR-associated risk factors include use of sunscreen, sunbathing vacations and use of indoor tanning devices.[30,31] Findings from the Norwegian Women and Cancer (NOWAC) study indicate that users of sunscreen report increased sunburns, sunbathing vacations and use of indoor tanning devices compared to non-users.[32] The use of sunscreen increased among Norwegian women during 1997–2007, both in high and low latitudes.[33] The use of sunscreen likely facilitates UVR exposure, increasing further with higher sun protection factor (SPF). Despite the increased use of sunscreen, it is estimated that a majority of people only apply 20–50% of the recommended amount and that reapplication is frequently neglected, resulting in sunburns.[34] However, the use of sunscreen with SPF ≥15 is associated with a decreased risk of melanoma compared to use of sunscreen with SPF <15.[32] Results such as these suggests that sunburn protection through appropriate sunscreen use is an important preventative measure against melanoma. The effectiveness of sunscreen use, while effective in experimental studies, remains heterogeneous and inconsistent in observational studies on humans, though the only randomized controlled trial showed protective effects. This is likely due to the challenges in controlling for innate confounding by indication.[35]

Indoor tanning includes the use of both private and commercial tanning devices (referred to as tanning beds or solariums), in which 95–100% of the body surface is exposed to UVR. This is a larger proportion compared to outdoor sun exposure, where 15–50% of the body surface is typically exposed to UVR.[36] While the proportions vary depending on the model of tanning bed lamp, most of the UVR range is from the UVA band (~95%), while only a small percentage (~5%) is UVB.[37-40] As opposed to outdoor sun exposure though, tanning devices deliver comparatively higher erythematic doses of UVR. The mean irradiance from solariums is 3.5 and 1.5 times higher for UVA and UVB, respectively, when compared to the UVR output of the summer sun in Oslo, the capital of Norway.[41,42] Use of indoor tanning increases the accumulated dose of UVR exposure and the high dose increases the risk of burning episodes. In 2009, the use of solariums was classified as carcinogenic to humans by the International Agency for Research on Cancer (IARC).[16] The use of indoor tanning devices is shown to have a positive dose-response relationship with an increased risk of melanoma, and increases the chance of an earlier melanoma diagnosis if exposed at a younger age.[43] The consensus is that use of indoor tanning is associated with a cumulatively increased

risk of melanoma, particularly in women, and that newer tanning technology has not improved safety.[30,44,45]

Sun exposure is an established cause of skin cancer and this has been communicated to the public since the early 1990s, and indoor tanning since 2009.[16] Measures taken in this regard include: educational campaigns focusing on prudent sun exposure behaviors, advertising efforts by manufacturers of sunscreen, protective clothing, and most recently the government measures to curb the use of tanning beds.[46] Despite these measures, frequent sunbathing combined with insufficient or wrong use of sunscreen remains a significant source of UVR exposure for all age groups, while indoor tanning primarily affects younger age groups. Norway recently enforced a previously imposed 18-year age-limit on the use of indoor solariums and results have in the short-term not shown a reduced use of solariums among 15–17-year-olds.[47] A similar study in the USA however, has demonstrated that age restriction laws can reduce indoor tanning prevalence among high school students when subjected to long-term follow up.[48]

1.3.2 Host pigmentation factors and nevi

Aside from UVR exposure, host factors influence the melanoma risk, specifically those factors related to individual susceptibility. These factors may additionally affect each individual's response to UVR exposure, and primarily include pigmentary characteristics [13], a previous melanoma diagnosis [49], and a family history of melanoma.[50] The results of current research efforts also suggest that there is a certain amount of interplay between environmental and host genetic factors that contributes to melanoma risk.[3]

Pigmentation characteristics include hair, skin and eye color, as well as number and type of nevi, and the potential to tan as a response to UVR exposure. It is established that fair-skinned individuals are at greater risk of developing melanoma, compared to individuals with darker skin pigmentation. This risk decreases along with increasing skin pigmentation profiles.[51] The Fitzpatrick skin phototype scale is commonly used to describe and classify a person's skin type in terms of its response to UVR exposure. Skin photoype is graded from I–V, where I is pale and burns easily, while phototype V is darkly pigmented and very resistant to tanning.[52] Compared to skin phototype IV, the relative risk of melanoma has been estimated to be 2.27, 1.99 and 1.35-fold higher for skin phototypes I, II and III respectively.[51] In a systematic review of 10 case-control studies, it was also estimated that persons with light skin color have a twofold increased risk of melanoma, compared to those with darker skin color.[53] Non-white persons have likewise a 10–20-fold decreased risk of melanoma when compared to white persons.[54] Melanoma has also been tied to blonde or red hair, as well as blue or green eyes. However, their merits as independent risk factors is questionable as such characteristics are often correlated with the level of skin pigmentation.[51]

Nevi are abnormal, but usually benign accumulations of melanocytes that can appear on any body location. The number of nevi is positively associated with melanoma risk [55,56], and is considered the most influential host risk factor for melanoma, with the risk being almost seven times higher in individuals with 101-120 nevi, compared to those with <15 nevi.[55] Melanoma risk also depends on the size and type of nevi present. Atypical and dysplastic nevi are more uncommon, except in those with atypical mole

syndromes, and are typified by a range of cytological irregularities, erythema, as well as color variations and large sizes.[13]

The presence of just five or more atypical nevi is enough to make melanoma risk over six times higher than in those without any atypical nevi.[55] The development of nevi as a determinant of melanoma is believed to be tied to both individual host genetic factors and UVR exposure patterns.[3] This may depend on the host's genetic propensity to develop nevi. This difference could in part explain the diverse body sites and risk factors associated with melanoma.[3]

1.3.3 Other associated factors

Individuals with a previous melanoma diagnosis were in a Norwegian study, found to have an 8-fold increased risk of a second melanoma.[49] A nationwide population-based study from Sweden has shown that individuals with a family history of melanoma (in parents or siblings) have a 2-8-fold increased melanoma risk.[50] In addition to the above-mentioned biological host factors, melanoma is a condition which also depends on the influence of a number of lifestyle factors. Factors suggested to elevate melanoma risk includes alcohol consumption[57] and body anthropometric factors such as large body surface area and high body mass index.[58] Positive associations between melanoma and high versus low physical activity is also observed, this may however, be explained by residual confounding due to UVR exposure.[59] Female sex hormones are also suggested to be related to melanoma risk.[60] While epidemiological findings have been inconsistent [61], a recent meta-analysis found an increased melanoma risk in users of oral contraceptives and hormone replacement therapy, as well as with parity (number of children/age at first childbirth).[62] However, residual confounding by factors related to parity, such as socioeconomic status and sun exposure factors cannot be ruled out.[61,62]

1.4 Pharmaceutical drug use and melanoma risk

Another factor with the potential to influence the risk of melanoma, is the increasing use of prescribed drugs. The systematic drug safety measures and active surveillance of adverse drug effects are highly prioritized within the European Medicines Agency database of suspected adverse drug reactions (EU-ADR). However, due to small follow-up cohorts and a lack of long-term monitoring in drug programs, the EU-ADR is not ideal for detecting outcomes with long latency periods, like most cancers. Similar limitations apply for the measures employed by the US Food and Drug Administration. For this reason, knowledge regarding the carcinogenicity of marketed pharmaceutical compounds is sporadic or lacking relative to their number.

The body of evidence concerning the association between pharmaceutical drug use and cancer, including melanoma, is largely based upon pharmacoepidemiological studies. IARC has been at the forefront in this regard, performing comprehensive and systematic reviews of animal, laboratory, mechanistic and epidemiological studies to evaluate the carcinogenicity of drugs since 1970. Group 1 agents are those considered carcinogenic to humans, whereas groups 2a and 2b are agents with probable and

possible carcinogenic effects, respectively.[63] Despite these efforts, many common drugs have not been formally evaluated and classified due to the aforementioned lack of long-term monitoring.

All drugs are classified according to the World Health Organization (WHO) Anatomical Therapeutic Chemical (ATC) classification system (version 2017). This system classifies drugs according to the organ or system upon which the active substance acts, as well as their chemical, pharmacological and therapeutic properties. Drugs are hence divided into five levels: main anatomical or pharmacological group (level 1), pharmacological or therapeutic subgroup (level 2), chemical, pharmacological or therapeutic subgroup (level 3 and 4) and chemical substance (level 5).[64]

Some drugs have the potential to induce or otherwise increase the risk of skin cancers.[65-67] The biological mechanisms behind such an effect, may include functional alterations of the immune system and the tumor microenvironment, and/or through an interaction with UVR exposure, resulting in photosensitivity reactions.[68,69] Antidepressant (ATC: N06A), immunosuppressant (ATC: L04), systemic corticosteroid (ATC: H02) and cardiovascular agents (ATC: C01–10) are prominent drug groups containing compounds with the potential to influence melanoma risk through such mechanisms.[65-67] In addition to a wide variety of effect mechanisms (particularly for cardiovascular drugs) they feature an expanding user base, possibly making the use of such drugs a significant contributing factor to the increasing melanoma rates. From 2004 to 2015, the number of users in Norway prescribed cardiovascular drugs rose by 37.0% (excluding inpatient use). The same numbers were 15.3% for antidepressants, 154.8% for immunosuppressants, and 42.0% for systemic corticosteroids (Table 1).[70]

Table 1: The number of users of prescribed antidepressant, immunosuppressant, systemic corticosteroid and cardiovascular drug compounds dispensed and recorded in the Norwegian prescription database in 2004 and 2015.[70]

Drug type	ATC	Number of users	
		2004	2015
Antidepressants	**N06A**	**279,816**	**322,652**
Non-selective monoamine reuptake inhibitors / Tricyclic antidepressants	N06AA	57,596	68,308
Selective serotonin reuptake inhibitors	N06AB	173,331	185,852
Monoamine oxidase inhibitors, non-selective	N06AF	147	88
Monoamine oxidase a inhibitors	N06AG	1621	721
Other antidepressants	N06AX	86,501	107,673
Immunosuppressants	**L04**	**24,002**	**61,160**
Selective immunosuppressants	L04AA	2689	9547
Tumor necrosis factor α inhibitors	L04AB	3571	15,157
Interleukin inhibitors	L04AC	72	1408
Calcineurin inhibitors	L04AD	3904	5974

Other immunosuppressants	L04AX	18,568	41453
Systemic corticosteroids	**H02**	**134,014**	**231,187**
Mineralocorticoids	H02AA	1019	1442
Glucocorticoids	H02AB	133,766	230,760
Corticosteroids for systemic use, combinations	H02BX	302	418
Cardiovascular drugs	**C01–10**	**785,480**	**1,076,122**
Cardiac therapy	C01	140,835	108,851
Antihypertensives	C02	26,581	16,812
Diuretics	C03	193,563	180,692
Peripheral vasodilators	C04	2377	653
Vasoprotectives	C05	52,319	71,852
Beta blocking agents	C07	309,765	372,948
Calcium channel blockers	C08	180,344	241,818
Agents acting on the renin-angiotensin system	C09	364,372	576,798
Lipid modifying agents	C10	306,139	530,137

Most studies highlight the need for further analyses with more detailed information regarding drug use and potential confounders to elucidate the associations between these drug types and cancer.[65] The fact that an increasing number of drug users has accompanied the high rate of melanoma in Norway, and that pharmacoepidemiological associations with melanoma and many common drug types remain unexplored, makes this an interesting and important subject for analysis for promoting safer and more informed drug use in the population.

1.4.1 Antidepressant drugs

Indication and mechanism of action

Antidepressants are prescribed primarily for the treatment of major depression, but also for psychological conditions like anxiety, obsessive-compulsive disorder, eating disorders, and post-traumatic stress disorder. The number of users of antidepressant drugs has been steadily increasing in many countries over the course of the last 50 years.[71,72] The major types of antidepressants include the tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs), and the newer serotonin reuptake inhibitors (SSRIs). While some uncertainty remains with regard to the molecular targets through which antidepressant agents exert their effect, the most prevalent hypothesis is that they relieve depression by gradually increasing the inter-synaptic availability of serotonin and norepinephrine by targeting neurotransmitter transporters.[73,74] The first generations of antidepressant drugs included the TCAs and MAOIs, which while clinically effective, induced several unwanted side effects. This was due to non-selective inhibition of other receptor sites, resulting in symptoms such as weight gain, constipation and dizziness, or in the case of MAOIs, adverse and potentially lethal interactions with other drugs.[73] These earlier drug types have since been largely

replaced by more selective drugs like the SSRIs, which are becoming the preferred treatment option for major depressive disorders.[71,72] It has been suggested that stress constitutes a separate risk factor for carcinogenesis.[75] Depression is also an indicator of other negative health conditions that may influence melanoma risk, such as increased alcohol consumption and weight gain.[76,77] A comprehensive study of potential risk factors for all skin cancer identified stress, traumatic events and depression as risk factors for melanoma.[78] While this may be a reflection of the resultant biological effects of such conditions or through lifestyle factors, it may also be indicative of associated antidepressant drug use.

The use of antidepressants and its carcinogenicity

Earlier studies on antidepressants found potential cancer promoting effects.[67,79] Recent results from other pre-clinical and animal studies however, have found that antidepressants, particularly TCAs, exert an inhibitory effect on the growth of *in vitro* tumor cells, including Burkitt lymphoma, colon, glioma, bladder and melanoma cell lines.[80-86] It is theorized that antidepressants may affect pathways which inhibit the malignant cell cycle and activates the immune system in ways that trigger apoptosis in cancer cells.[84-86]

As with pre-clinical studies, a large number of epidemiological studies have, in recent decades, also investigated the association between antidepressant use and several cancer types. Earlier studies indicated that long-term TCA and SSRI antidepressant exposure increased the risk of cancer, particularly breast, colon and ovarian cancer.[85] Long-term use of TCAs has also been linked to an increased risk of non-Hodgkin lymphoma and prostate cancer, though the evidence for this was not clear.[85] Later studies on the subject however, have consistently not attributed any excess cancer risk to antidepressant use. The risk of colon and lung cancer was in fact shown to be reduced among users of SSRIs.[85] Recent epidemiological studies on the subject have also found no associations between antidepressant use and breast cancer, including a decreased risk of epithelial ovarian cancer.[87,88] However, cancer promoting effects have also been observed and thus the relationship is not clarified.[85,89,90] It is also worth noting that the majority of these studies vary in terms of design and methodology. Relatively few studies also focus on SSRIs, the antidepressants most commonly prescribed. Large-scale observational studies regarding the association between varied antidepressant drug use and risk of melanoma are currently lacking in the literature.

1.4.2 Immunomodulating drugs

Indication and mechanism of action

Immunomodulating drugs refers in the case of this study, to both immunosuppressants (ATC: L04) and systemic corticosteroids (ATC: H02) (hereafter corticosteroids). Immunomodulating drugs are agents which positively or negatively affect the immune system in a direct or indirect way. These drugs are commonly prescribed alone or in combination as primary treatment for a range of inflammatory, autoimmune and immune-mediated diseases. They are also used to maintain transplanted organs and prevent an immune-mediated rejection.[91,92] Our understanding of immune system

mechanisms has progressed rapidly over the last decades due to mechanistic research and numerous immunosuppressive drug discoveries.[92] Early immunomodulating drugs displayed many well-documented side-effects and toxicities, which is thought to be due to the non-specific nature of their immune-modulation. This has since prompted the development of an array of modern immunomodulating drugs with improved specificity and efficacy, especially following the discovery of the landmark cyclosporine A and the development of humanized monoclonal antibodies in the 1990s.[92]

The use of immunomodulating drugs and its carcinogenicity

The progression and regression of melanoma is regulated partly by immune system mechanisms, and its development may be affected by long-term exposure to immunomodulating drugs.[93] The use of tumor necrosis factor- α inhibitors in patients with rheumatoid arthritis has previously been linked to an increased risk of melanoma,[94] although not confirmed in a more recent international multi-register study.[95] Interleukin inhibitors are another class of immunosuppressants where use is associated with both melanoma and KC.[96-98] Methotrexate, a widely used immunosuppressive drug, has been associated with increased melanoma risk and mortality[99-101], though evidence for a dose-response relationship is lacking.[102]

Among the side effects associated with the use of immunosuppressants, post-transplant infections and cancers are some of the most frequent and severe. Among immunocompromised populations, organ transplant recipients (OTRs) are known to be subject to a particularly increased risk of both melanoma and KC.[66,103-108] Furthermore, certain immunosuppressants have a documented ability to exacerbate UVR–induced DNA damage, which is theorized to be responsible for a potential elevated risk of melanoma.[109,110] Some immunosuppressants, like azathioprine, are thought to stimulate photosensitivity in long-term users. In synergy with UVR exposure, these agents precipitate DNA damage through the production of reactive oxygen species (ROS) with mutagenic potential.[109] This is thought to stimulate the development of melanoma and KC.[110,111]

Corticosteroids are primarily used alone or in combination with other immunomodulating drugs as an anti-inflammatory and immunosuppressive therapy for disease typified by acute or chronic inflammation (e.g. rheumatoid arthritis, psoriasis, inflammatory bowel syndrome and eczema).[112] Of all systemic corticosteroid users in Norway, glucocorticoid (ATC: H02AB) users made up 99.8% in both 2004 and 2015, making them by far the most commonly prescribed of all systemic corticosteroids.[70] Pre-clinical studies have linked glucocorticoid exposure to an inhibition of human melanoma cells.[113-115] Yet, its use has been associated with numerous, often carcinogenic side effects, as well as an increased risk of KC.[116-119] Despite its widespread use, observational studies concerning the association between melanoma and corticosteroids are lacking.

1.4.3 Cardiovascular drugs

Indication and mechanism of action

Cardiovascular agents belong to a large group of drugs with varied modes of efficacy, of which all are aimed at treating conditions associated with the heart or blood circulatory system. This includes many commonly occurring conditions in industrialized countries, such as infarction, arrhythmia, abnormal blood pressure, high cholesterol and stroke. For this reason, cardiovascular drugs remain some of the most widely used and developed drug groups. In Norway, the number of individuals using these drugs exceeded 1 million by 2015.[70] The largest group of these drugs is the antihypertensives, which includes diuretics, beta blocking agents, calcium channel blockers and agents acting on the renin-angiotensin system (RAS) (Table 1).[70] Diuretics are commonly prescribed for cases of heart congestion and hypertension, and function primarily by preventing the reabsorption of sodium by inhibiting various ion channels or transporters within the nephrons. Beta blockers are similarly prescribed for heart congestion, hypertension and arrhythmia, and function by blocking the effect of epinephrine and norepinephrine by inhibiting the $\beta 1$ and/or $\beta 2$ receptors. Calcium channel blockers are often prescribed for hypertension, angina and arrhythmia and block the uptake of calcium in the heart or blood vessels by inhibiting the respective L- and T-type voltage-gated calcium channels. The RAS regulates blood pressure and its drug agents primarily include the angiotensin converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARBs). ACEi agents prevents angiotensin I from converting to angiotensin II, while ARBs prevents angiotensin II function by inhibiting angiotensin II receptors.[120]

Photosensitivity can be defined as an abnormal or inappropriate response to UVR, a factor previously described in this thesis to influence the risk of melanoma. This effect depends on the particular drug and its dose, and can often go unnoticed by the affected person due to its subclinical nature and similarities to sunburn symptoms.[121] The photosensitizing potential of a drug depends on the capability of its chemical structure to absorb UVR.[122,123] In synergy with UVR, the drug may cause a photosensitive dermatological reaction. This may manifest shortly after drug administration as a phototoxic reaction, in which the generation of ROS due to UVR exposure causes acute DNA-damage. Alternatively, a photoallergic reaction may be elicited after a latent period of days or months following drug administration and is typically more severe. The latter reaction is caused by the formation of UVR-induced antigens which triggers a T-cell driven immune reaction.[123]

Almost all cardiovascular drug classes have reported photosensitivity reactions and contain the third-highest number of such compounds, after nervous system and anti-infectious drugs (Table 2).[124] The sub-groups of cardiovascular drugs which contain compounds with photosensitizing potential include cardiac therapy (ATC: C01), antihypertensives (ATC: C02), beta blocking agents (ATC: C07), calcium channel blockers (ATC: C08), lipid-modifying agents (ATC: C10), diuretics (ATC: C03) and agents acting on the RAS (ATC: C09), of which the two latter contain the highest number of such drugs.[124-127] When considering the implications for an outcome like melanoma, it is prudent to consider the large number of cardiovascular drugs with photosensitizing potential.[125-127] On the other hand, as tumor growth requires a steady

blood supply, its inhibition by cardiovascular agents has been suggested to contribute to the inhibition of cancer.[120,128]

The use of cardiovascular drugs and its carcinogenicity

The use of several diuretics has been linked to an increased risk of melanoma and SCC.[120] A meta-analysis of observational studies also found a positive association for melanoma.[129] Drug-induced photosensitivity remains a clinical problem with certain diuretic drugs and may partly explain the positive association.[69,130] This particularly concerns hydrochlorothiazide and to a lesser extent furosemide, which are both capable of eliciting various dermatological reactions, including photosensitivity.[127,130] Regarding the diuretic agent hydrochlorothiazide, no general association was discovered for melanoma in a Danish nation-wide study, though when stratified by histological subtype, it was associated with an increased risk of both lentigo and nodular melanoma.[131] The strongest associations have indeed been found with thiazide diuretics, including hydrochlorothiazide.[132-134] However, the association between diuretics overall and melanoma is weak, and another meta-analysis found no associations with melanoma risk.[65] Likewise, a recent review concluded that the use of diuretics overall is currently not considered a risk factor for melanoma.[120]

Meta-analyses of epidemiological studies have found that users of beta-blocking agents are subjected to an increased risk of melanoma.[65,129] On the other hand, long-term use of beta-blocking agents has been associated with reduced risk of melanoma progression[135,136], recurrence and death.[136-138] A recent review also concluded that use of beta-blockers overall, does not increase the risk of melanoma, but can rather improve survival and may synergize as an adjunctive to immunotherapies.[120]

The epidemiological findings for use of calcium channel blockers have indicated that it is not generally associated with the incidence, recurrence or mortality of any cancer [139-141], yet more recent reviews and meta-analyses show conflicting results.[65,129] Findings of *in vitro* drug studies also suggest that they may have a synergistic role in the treatment of tumors, even melanoma.[120] Of the calcium channel blocking agents dispensed in Norway however, the use of nifedipine, nimodipine and diltiazem have all been associated with photosensitivity reactions, while other adverse dermatological effects have been observed in amplodipine and verapamil as well.[126,130,142]

ACEi and ARBs contain several agents with documented dermatological side-effects, including photosensitivity reactions. This includes agents such as the ACEi captopril, enalapril, lisinopril and the ARB losartan, all of which feature thousands of users in Norway.[70,127,130] Meta-analyses of observational and experimental studies have found that use of ACEi or ARBs is not associated with an increased risk of skin cancer or melanoma.[65,129] A recent review however, concluded that while experimental studies indicate a possible therapeutic role for ACEi and ARBs, epidemiological studies show that long-term use may increase the risk of melanoma. A finding which may be explained by their possible photosensitizing effect.[120]

Table 2: The number of users of pharmaceutical cardiovascular drug compounds dispensed and recorded in the NorPD in 2004 and 2015 with photosensitizing potential (excluding inpatient use).[70,124]

Photosensitizing cardiovascular drugs	ATC	Number of users	
Cardiac therapy	**C01**	**2004**	**2015**
Quenidine	C01BA01	25	<5
Disopyramide	C01BA03	254	89
Amiodarone	C01BD01	3223	5977
Dronedarone	C01BD07	0	1924
Antihypertensives	**C02**		
Methyldopa	C02AB01	1284	57
Hydralazine	C02DB02	235	312
Diuretics	**C03**		
Bendroflumethiazide	C03AA01	13,002	0
Hydrochlorothiazide	C03AA03	7719	12,986
Chlortalidone	C03BA04	5	7
Metolazone	C03BA08	<5	7
Furosemide	C03CA01	115,758	82,920
Bumetanide	C03CA02	15,899	41,453
Torasemide	C03CA04	<5	<5
Spironolactone	C03DA01	15,459	18,788
Amiloride	C03DB01	28	23
Beta blocking agents	**C07**		
Propranolol	C07AA05	17,428	15,732
Sotalol	C07AA07	14,002	5 228
Carvedilol	C07AG02	23,630	19,308
Calcium channel blocking agents	**C08**		
Amplodipine	C08CA01	103,527	131,271
Nifedipine	C08CA05	22,208	42,081
Verapamil	C08DA01	22,157	12,400
Diltiazem	C08DB01	9446	3 941
Agents acting on the renin-angiotensin system	**C09**		
Captopril	C09AA01	5 840	1531
Enalapril	C09AA02	42,956	45,614
Lisinopril	C09AA03	32,597	22,225
Ramipril	C09AA05	37,777	65,906
Losartan	C09CA01	44,500	65,516
Valsartan	C09CA03	17,952	32,317
Irbesartan	C09CA04	24,476	18,442
Candesartan	C09CA06	41,043	102,198
Telmisartan	C09CA07	1531	5651
Olmesartan medoxomil	C09CA08	0	1 553

2. Aims of thesis

2.1 General aim

The main aim of this thesis was to investigate the associations between the use of antidepressant, immunomodulating and antihypertensive drug groups and melanoma risk. The thesis also aimed to investigate these associations based on person characteristics (sex, age at diagnosis/index date and residential ambient UVR exposure), and clinical melanoma characteristics (melanoma site, histopathological subtype and clinical stage at diagnosis).

The papers included are a protocol paper (0), and three research papers (I–III). Paper 0 presents the general aspects of the data, study design and exposure assessments for each of the three subsequent research papers. Papers I–III investigate the associations between the use of prescribed antidepressant (N06A), immunomodulating (L04 & H02) and antihypertensive (C03, 07–09) drugs and melanoma risk, respectively.

2.2 Specific aims

The specific aims of the papers were:

Paper 0

- To describe the study design, data collection, exposure assessment and the statistical methods in the three subsequent research papers.

Paper I

- To examine the association between the long-term use and cumulative use of prescribed antidepressant drugs (N06A) and melanoma risk.
- To evaluate the impact of sex, age, residential ambient UVR exposure, anatomic site, histopathological subtype and clinical stage on this association.

Paper II

- To examine the association between the long-term use and cumulative use of prescribed immunomodulating drugs (L04 & H02) and melanoma risk.
- To evaluate the impact of sex, age, residential ambient UVR exposure, anatomic site, histopathological subtype and clinical stage on this association.

Paper III

- To examine the association between the long-term use and cumulative use of prescribed antihypertensive drugs (C03, 07–09) and melanoma risk, with an added focus on the impact of photosensitizing agents.
- To evaluate the impact of sex, age, residential ambient UVR exposure, anatomic site, histopathological subtype and clinical stage on this association.

3. Materials and methods

3.1 Study design

We performed a matched nested case-control study. The data set comprised all first primary diagnoses of cutaneous melanoma in the Norwegian population in 2007–2015 as registered in the CRN, along with ten controls matched to each case on sex and age at diagnosis date as identified by the Norwegian National Registry (NNR) (Table 3). For all individuals, data on drug prescriptions filled in 2004–2015 was received from the Norwegian Prescription Database (NorPD) and data on parity (women only) from the Medical Birth Registry of Norway (MBRN) (Figure 5). Data from each registry was linked using the unique personal identification numbers (PIN) assigned to all residents in Norway.
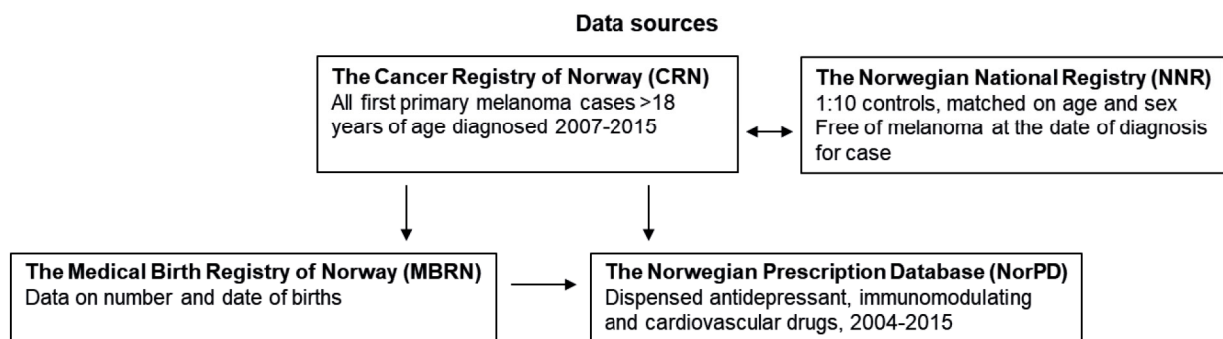
Data sources

Figure 5. Data sources and data flow in the nested case-control study.

3.2 Data collection and categorization of variables

3.2.1 The Cancer Registry of Norway (CRN)

The CRN has been compelled by law to record data regarding cancer diagnoses in Norway since 1953. The CRN relies on several independent sources for its data, including medical practitioners, pathology laboratories and the Norwegian Cause of Death Registry. This ensures complete and high quality data. Since 2000, over 99% of all melanoma cases have been morphologically verified.[12,143] For the present study, we obtained data on all individuals with a first primary morphologically verified cutaneous melanoma diagnosis during 2007–2015 (n=12,877), according to the International Classification of Diseases (ICD) of oncology 3rd edition (ICD-O-3) and the ICD 10th Revision (C43). We obtained information on sex (men/women), date of birth (month/year), date of diagnosis (month/year), melanoma site, histological subtype, clinical stage of disease and county of residence.

Tumor site was categorized as head/neck (C43.0–4), trunk (C43.5), upper limb (C43.6), lower limb (C43.7), other (C43.8) and unspecified (C43.9). Histological subtype was categorized as superficial spreading melanoma (SSM; 87433), nodular melanoma (NM; 87213) and other subtypes (87423, 87443, 87453/87803/87613, including unspecified 87203). Based on information about metastases, CRN records

stage at diagnosis as local disease (no metastases), regional metastasis (metastases in regional lymph nodes, satellites and in transit metastases), distant metastasis (organ metastases and non-regional lymph node metastases) and unspecified. The county of residence for each case was used to categorize the level of ambient UVR exposure into a five-level variable (northern Norway, central Norway, southwestern Norway, southeast inland, and southeast coast).[20] This was also collapsed into a three–level variable (low - northern Norway, medium - western and central Norway and highest - eastern and southern Norway).[23]

3.2.2 The Norwegian National Registry (NNR)

The NNR records information on vital status for all residents in Norway. This allows the sampling of general population controls for all melanoma cases. Risk set sampling was used to select controls at random (with replacements) from the NNR on a 1:10 basis for each melanoma case, matched on sex and year of birth (n=128,768). Controls had to be alive, reside in Norway and free of any previous cancer (except for BCC) at the date of diagnosis for their respective cases (index date), though could develop cancer thereafter (Table 3).

Table 3: Overview of case, control and matching criteria for the study sample.

Cases	Study criteria
Number of cases	12,877
Verification	Histological or cytological verified melanoma (ICD-10: C43)
Definition	Resident in Norway with a diagnosis of invasive melanoma without a history of cancer
Age at diagnosis	18–85 years
Year of diagnosis	2007—2015
Sex	Male and female
Controls	
Number of controls	128,768 (1:10 matching)
Definition	Alive and resident in Norway with no history of cancer before respective case diagnosis
Selection	Random sampling within matching criteria (with replacements) from a pool of available population
Matching criteria	
Sex	Same sex as case
Year of birth	Same year of birth as case
Index date	Alive and cancer-free at date of diagnosis (case)

3.2.3 The Norwegian Prescription Database (NorPD)

The NorPD has recorded all prescribed drugs dispensed nationwide in Norwegian pharmacies to non-institutionalized individuals and outpatients since 1 January 2004 (reimbursed or not).[144] For each record of dispensation, the NorPD records the date of dispensation, and information regarding the identity of the patient and prescriber. The NorPD also records information regarding the drugs themselves, including substance name, trade name, pharmaceutical formulation and amount dispensed in defined daily doses (DDD), which is defined as the assumed average maintenance dose per day for

a drug used for its main indication in adults. All drugs are classified according to the WHO ATC classification system (version 2017).[64]

From the NorPD, we obtained data regarding antidepressant (ATC: N06A), immunomodulating (ATC: L04 & H02) and cardiovascular (ATC: C01–10) drug prescriptions for all cases and controls, including the number of prescriptions of any other drugs from 2004–2015. We also obtained county of residence from the NorPD for all controls, which was categorized as described above for the cases.

3.2.4 Categorization of drug use

From the NorPD, we obtained data regarding the patient, the prescriber and the drug for each dispensation, including the corresponding ATC classification code, the number of DDD dispensed and the date of dispensation (month and year).[144] To mitigate the potential risk of reverse causation bias, prescriptions of any drug dispensed within a year prior to the diagnosis/index date were disregarded in all analyses.

- Antidepressants were defined as any drug included in the ATC group N06A, and were classified according to ATC 4th level as selective serotonin reuptake inhibitors (SSRI; N06AB), tricyclic antidepressants (TCAs; N06AA) and other antidepressants (N06AF, N06AG and N06AX). Use of antidepressants from more than one such class was defined as mixed use.
- Immunosuppressants were defined as any drug included in the ATC group L04. Due to the elevated skin cancer risk reported for OTRs and because the most commonly prescribed immunosuppressant was methotrexate (L04AX03), immunosuppressant drugs commonly prescribed to OTRs (L04AA06/10/18, L04AD01/02)[145] and methotrexate (L04AX03) were analyzed separately. Users of all remaining immunosuppressants constituted the group "other drugs with immunosuppressant actions" (L04AA13/21/24/27/31, L04AB01/02/04/05/06, L04AC03/05, L04AX01/02/05). Corticosteroids were defined as any drug included in the ATC group H02, and were analyzed as one group due to the many different and overlapping indications for use.[146]
- Cardiovascular drugs were defined as any drug included in the ATC group C and were classified according to ATC 2nd level, focusing on the most frequently prescribed antihypertensive drug types in Norway and the world. These included diuretics (C03), beta blockers (C07), calcium channel blockers (C08) and agents of the RAS (C09).

Number of prescriptions

The total number of prescriptions of each drug group was quantified for each case and control based on the total number of prescriptions filled from 1 January 2004 up until a year prior to diagnosis/index date. The number of prescriptions was categorized as: 0–1 prescription (non-use), 2–7 prescriptions and ≥8 prescriptions, for all antidepressant drug groups in paper I, as well as all immunomodulating drugs, except methotrexate in paper II. This categorization was based on the assumption that each drug prescription is equivalent to 3 months of use.[147] For the immunomodulating drug methotrexate in paper II, the number of prescriptions was categorized as 0–1

prescription, 2-3 prescriptions and ≥4 prescriptions, due to each prescription usually lasting for 6 months.[145] The number of all antihypertensive drug prescriptions in paper III were categorized as 0–1 prescription and ≥2 prescriptions due to the small number of persons in the 2–7 prescription category. Any prescriptions in papers I–III may have been obtained at separate or the same dates.

Cumulative dose

The cumulative dose of any drug group was quantified for each case and control based on the total number of DDDs for each prescription. In paper I, the cumulative dose of antidepressant drugs was categorized as non-users (0 DDD) and in quartiles (1–91; 92–365; 366–1460; ≥1461 DDD) based on the whole study sample, for antidepressants overall and for each class of antidepressant.

The categories of use for immunomodulating drugs in paper II corresponded to <1, 1-3, 4-5, 6-8 and >8 years of use, as such drugs are typically prescribed for long-lasting indications.[92,112] Thus, for immunosuppressant and corticosteroid drugs overall the cumulative dose was categorized as non-users (0 DDD) and in user levels (1-365; 366-1100; 1101-1800; 1801-2900; ≥2901 DDD). For the subgroups of immunosuppressants, drugs prescribed to OTRs, methotrexate and "other drugs with immunosuppressant actions", DDD user levels were categorized as: 1-365; 366-1800; ≥1801 DDD, due to a low number of drug users in certain categories.

In paper III, for cumulative dose of antihypertensive drugs, we categorized non-use as 0 DDD and use as the total number of DDDs categorized in quartiles for each antihypertensive drug type/group (diuretics 1-197, 198-699, 700-2199, ≥2200; beta-blockers 1-188, 189-816, 817-1959, ≥1960; calcium channel blockers 1-459, 460-1799, 1800-4065, ≥4066; and RAS agents 1-1187, 1188-3037, 3038-4923, ≥4924).

Duration of use

For paper I, duration of use of antidepressants overall was defined based on time between the first and last antidepressant drug prescription, and was categorized as non-user (no prescriptions during 2004-2015), ≤5 years (which can include only one prescription) and >5 years. This analysis was not repeated for paper II or III as the analyses of cumulative dose made it unnecessary.

Adjustment for other drug use

In the analyses of associations between drug use and melanoma risk in papers I-III, information about drug use, other than the drug in focus, was used as a covariate in the analysis model. Use was categorized as ever use of antidepressants (yes/no), ever use of immunomodulating drugs (yes/no), ever use of cardiovascular drugs (yes/no) and >1 prescription filled of any other drugs (yes/no).

3.2.5 The Medical Birth Registry of Norway (MBRN)

The MBRN was established in 1967 and has since recorded information on all births in Norway. To consider the potential influence of parity on the association between the

respective drugs and melanoma risk, information about number and dates of births experienced up until the point of diagnosis/index date was obtained for all female cases and controls from 2004. Parity (number of children) prior to diagnosis/index date was categorized as 0, 1–3 and >3 children.

3.3 Study population

Initially the dataset consisted of 141,645 persons, with data collected and merged from the CRN, NorPD, NRR and MBRN (12,877 melanoma cases and 128,768 controls) (Table 3). The study populations for papers I-III, with inclusion/exclusion criteria, are illustrated in Figure 6. Persons above 85 are more likely to reside in institutions like hospitals or nursing homes, where prescription data is not reported to the NorPD. Thus, we restricted the age at diagnosis to only include adult cases diagnosed at 18–85 years. Due to this restriction, we excluded 7650 persons (704 cases and 6946 controls) for papers I-II and 7832 persons (712 cases and 7120 controls) for paper III who were >85 years of age at diagnosis/index date (there were none <18 years of age at diagnosis/index date). We excluded 3324 persons (67 cases and 3257 controls) for papers I-II and 3100 persons (47 cases and 3053 controls) for paper III with no data concerning county of residence (from CRN or NorPD).

For paper I, 105 persons (7 cases and 98 controls) were excluded due to missing data on prescription dosage and conflicts between number of prescriptions and cumulative dose. Thus, the final study population for paper I consisted of 130,566 persons, of which 12,099 were melanoma cases and 118,467 were controls (Figure 6). For paper II we excluded 1 control with missing data on prescription dosage. Thus, the final study population consisted of 130,670 persons, of which 12,106 were melanoma cases and 118,564 were controls (Figure 6). For paper III, we removed an additional 70 previously undiscovered duplicate cases along with their respective controls (n=700) before excluding persons based on age and missing county of residence. Each such case represented an additional melanoma diagnosis for the same person and was thus registered as a separate observation by the CRN. After exclusions based on age and lack of region of residence the final study population for paper III consisted of 129,943 persons, of which 12,048 were melanoma cases and 117,895 were controls (Figure 6).
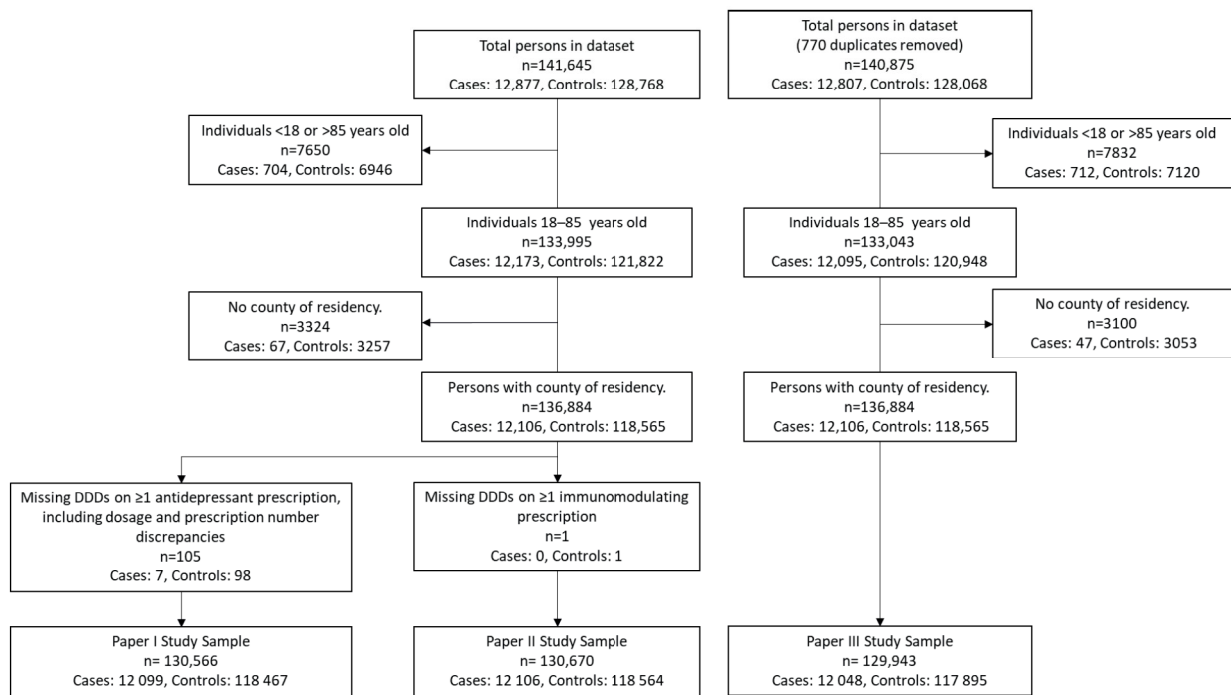
Figure 6. Illustration of study populations and inclusion/exclusion criteria for papers I, II and III

3.4 Statistical analyses

For papers I and II, all statistical analyses were performed using the statistical software R (version 3.5.1).[148] For paper III, all statistical analyses were performed using the statistical software Stata (version 16).

The significance level was set to 5% and all tests were two-sided.

In papers I-III, we used conditional logistic regression to investigate the associations between the use of a particular drug group and melanoma risk. The results are presented as estimated rate ratios (RRs) with 95% confidence intervals (CIs), since the selection of controls was done by risk-set sampling.[149] The controls were matched with respect to sex and age at diagnosis/index date and the analysis was conditioned on the risk sets. Moreover, we adjusted for residential ambient UVR exposure and other drug use in the multivariable regression models. Additional adjustment for parity for women did not change the effect estimates (results not shown).

In paper II, immunosuppressant (L04) and corticosteroid (H02) drug use were mutually adjusted. Separate analyses of OTR drugs and methotrexate were adjusted for corticosteroid drug use, including use of non-OTR and non-methotrexate immunosuppressive drugs, respectively. Analyses in paper III were additionally adjusted for use of non-antihypertensive cardiovascular drug types. Analyses based on number of prescriptions of a particular antihypertensive drug type separated between use in combination with other antihypertensive drug types (mixed use) and exclusive use. These analyses were also conducted with an active-comparator design [150], in which the non-use category only constituted users of other cardiovascular drug types. For example, non-users of diuretics were users of all other cardiovascular drugs except diuretics.

In paper I, the median number of drug prescriptions or DDD of each user category was used to test for trends in the number of prescriptions or DDD, respectively, for each antidepressant drug group. In addition, RRs were estimated for the association between melanoma and the duration of use with a short (≤5 years) and long (>5 years) timeframe, and for the associations between the histological subtypes of melanoma and number of prescriptions by antidepressant classes (supplementary material).

Interaction terms in the models were compared using a likelihood ratio test to test for statistical interactions between the number of drug prescriptions and sex, age (at diagnosis/index date), residential ambient UVR exposure (three level variable) and parity (women only) on melanoma risk (i.e. differences in effect estimates for the drug variable at different levels of these covariates). According to the aims, RRs were estimated for the associations between the number of antidepressant, immunomodulating and antihypertensive drug prescriptions, and melanoma risk, stratified by sex, age (at diagnosis/index date) and region of ambient UVR exposure. We also conducted analyses of disease subtype heterogeneity (melanoma site, histological subtype and clinical stage at diagnosis).[151] Tests for heterogeneity were performed by melanoma site, histological subtype and clinical stage at diagnosis in paper II, and by melanoma site and histological subtype in paper III using contrast tests (i.e. whether the exposure-disease association differs among the subtypes).

4. Results

4.1 Paper 0

Berge LAM, Andreassen BK, Stenehjem JS, Larsen IK, Furu K, Juzeniene A, Roscher I, Heir T, Green A, Veierød MB, Robsahm TE. *Cardiovascular, antidepressant and immunosuppressive drug use in relation to risk of cutaneous melanoma: a protocol for a prospective case-control study.* BMJ Open 2019; 9(2): e025246.

In this protocol paper, we explained the scientific background for conducting a study that aims to investigate the associations between use of the selected drug groups and melanoma risk. The paper describes the study design, the data and methods applied. It details the plan for investigating the association between melanoma risk and prescription drug use, while considering potential confounding factors. This paper has been the basis and an important framework for the decision-making and implementation of the thesis' three research papers. The publication of this paper also provided valuable scientific peer-review feedback before starting the work on papers I-III.

4.2 Paper I

Berge LAM, Andreassen BK, Stenehjem JS, Heir T, Furu K, Juzeniene A, Roscher I, Larsen IK, Green AC, Veierød MB, Robsahm TE. *Use of antidepressants and risk of cutaneous melanoma: A prospective registry-based case-control study.* Clin Epidemiol. 2020; 12:193-202.

Most cases were >50 years at date of diagnosis and 74.1% resided in the southern and eastern regions of Norway. Melanomas were most frequently located on the trunk and lower limb (47% and 24% of cases, respectively). The most commonly diagnosed histological subtype was SSM (55% of cases) and the majority of melanomas were diagnosed in a local stage (81% of cases). A higher proportion of women made up the users of ≥8 and 2–7 prescriptions (69% and 63% respectively). SSRI antidepressants were the most frequently prescribed among users overall (51%), of which escitalopram was the most common specific drug.

Users of ≥8 antidepressant prescriptions overall had a 19% decreased risk of melanoma, compared to non-users (≤1 prescription) (RR 0.81, CI 0.75–0.87). A trend of reduced melanoma risk was also seen with increasing user levels of antidepressant prescriptions (P_{trend}<0.001). An 18% lower risk of melanoma was found in exclusive users of ≥8 prescriptions of SSRIs (RR 0.82, 0.73–0.93) and 23% in users of mixed antidepressant classes (RR 0.77, CI 0.69–0.86). A negative trend of melanoma risk was found for increasing user levels of SSRIs (P_{trend}=0.003), other (P_{trend}=0.036) and mixed antidepressants (P_{trend}<0.001). Long-term users (>5 years) with 2-7 and ≥8 prescriptions had a reduced risk of melanoma (RR 0.52, CI 0.38–0.74). For short-term users (≤5 years), a reduced melanoma risk was observed for use of ≥8 prescriptions.

When investigating the cumulative dose of antidepressants overall, a 15% increased risk of melanoma was found for non-users (RR 1.15, CI 1.04–1.26), and a negative trend of melanoma risk was seen for increasing dose levels. In analyses stratified by antidepressant classes, a 65% and 69% increased risk of melanoma was found in

mixed users of 92–365 DDD (RR 1.65, CI 1.09–2.50) and in non-users (RR 1.69, CI 1.16–2.45), respectively, when compared to the lowest dose level (1-91 DDD).

Use of ≥8 prescriptions of antidepressants overall was associated with a reduced risk of melanoma in men (RR 0.73, CI 0.64–0.83) and to a lesser extent in women (RR 0.86, CI 0.78–0.94) ($P_{interaction}$=0.029). A reduced risk of melanoma was seen for people in the age group 50–69 years (RR 0.76, CI 0.68–0.85) and for those in the age group ≥70 (RR 0.79, CI 0.69–0.90) ($P_{interaction}$=0.013). A reduced risk of melanoma was also found in users of ≥8 prescriptions residing in the region with medium (RR 0.73, CI 0.55–0.95) and the highest (RR 0.82, CI 0.75–0.89) levels of ambient UVR exposure ($P_{interaction}$=0.379).

Use of ≥8 antidepressant prescriptions overall was associated with a decreased risk of melanoma for the trunk (RR 0.79, CI 0.70–0.88), upper (RR 0.80, CI 0.66–0.97) and lower limb (RR 0.76, CI 0.65–0.88) sites. A negative association was also seen across all three histological subtypes, (P_{trend}<0.001 (SSM); 0.017 (NM); 0.013 (other)). Supplementary analyses showed that ≥8 prescriptions of SSRIs were associated with a decreased risk of SSM (RR 0.81, CI 0.69–0.96). Use of mixed antidepressants was associated with a decreased risk of SSM (RR 0.75, CI 0.65–0.87) and NM (RR 0.70, CI 0.52–0.94). A reduced risk of local stage melanoma was seen in users of ≥8 prescriptions (RR 0.79 CI 0.73–0.86), though not for any other stages.

4.3 Paper II

Berge LAM, Andreassen BK, Stenehjem JS, Heir T, Karlstad Ø, Juzeniene A, Ghiasvand R, Larsen IK, Green AC, Veierød MB, Robsahm TE. *Use of immunomodulating drugs and risk of cutaneous melanoma: A prospective registry-based case-control study.* Clin Epidemiol. 2020; 12:1389-1401

The composition of cases with regard to residential ambient UVR exposure, body site, histological subtype and metastasis were the same as in paper I. Users of ≥8 prescriptions of immunosuppressant drugs had a 50% increased risk of melanoma (RR 1.50, CI 1.27–1.77) compared to non-users (≤1 prescription). Use of ≥8 prescriptions of OTR drugs was associated with a two-fold increased risk (RR 2.02, CI 1.35–3.03), while use of ≥4 prescriptions of methotrexate was associated with a 27% increased risk (RR 1.27, CI 1.04–1.55). For cumulative dose, only users of immunosuppressant drugs overall displayed an increased melanoma risk.

Men using ≥8 prescriptions of immunosuppressants had a 36% increased risk of melanoma (RR 1.36, CI 1.05–1.77), while the risk was 60% for women (RR 1.60, CI 1.29–1.98) ($P_{interaction}$=0.068). For corticosteroid use, a 20% reduced risk of melanoma was found in men (RR 0.80, CI 1.29–1.98) ($P_{interaction}$=0.018). Users of ≥8 prescriptions of immunosuppressants had a 47% and 72% increased risk of melanoma, for the age groups 50-69 (RR 1.47, CI 1.17–1.85) and ≥70 (RR 1.72, CI 1.29–2.29)), respectively ($P_{interaction}$=0.174). The melanoma risk was more than two-fold (RR 2.36, CI 1.26–4.45) for users of ≥8 prescriptions of immunosuppressants in the region of medium UVR exposure, with the risk being 52% in the region with the highest UVR exposure (RR 1.52, CI 1.26–1.85) ($P_{interaction}$=0.105). Finally, in the region of medium UVR exposure users of ≥8 prescriptions of corticosteroids had a 53% reduced risk of melanoma (RR 0.47, CI 0.27–0.81) ($P_{interaction}$=0.064).

Users of ≥8 prescriptions of immunosuppressants had a 67% and 51% increased risk of melanoma for the trunk (RR 1.67, CI 1.30–2.13) and upper limb (RR 1.51, CI 0.96–2.36), respectively, while more than a two-fold increased risk was found for the head/neck sites (RR 2.22, CI 1.45–3.40). An increased risk of melanoma was likewise found for use of ≥8 prescriptions of immunosuppressants across all histological subtypes. We also found a 50% increased risk of local stage melanoma (RR 1.50, CI 1.26–1.79) and more than a two-fold increased risk for melanoma with regional metastasis (RR 2.17, CI 1.04–4.54). However, the tests for heterogeneity showed no significant differences between the effect estimates for tumor site, histological subtype or clinical stage, for either immunosuppressants ($P_{heterogeneity}$=0.112, 0.676, 0.338, respectively) or corticosteroid prescriptions ($P_{heterogeneity}$=0.507, 0.417, 0.260, respectively).

4.4 Paper III
Ghiasvand R, Berge LAM, Andreassen BK, Stenehjem JS, Heir T, Karlstad Ø, Juzeniene A, Larsen IK, Green AC, Veierød MB, Robsahm TE. *Use of antihypertensive drugs and risk of cutaneous melanoma: A nationwide nested case-control study.*

The composition of cases with regard to residential ambient UVR exposure, body site, histological subtype and metastasis were the same as in papers I and II. Firstly, when comparing use (≥2 prescriptions) to non-use (≤1 prescription) for selected antihypertensive drug types, elevated risk of melanoma was found for users of diuretics (RR 1.08, CI 1.01–1.15), calcium channel blockers (RR 1.10, CI 1.04–1.18) and agents of the RAS (RR 1.10, CI 1.04–1.16). When analyzed by sex, the elevated risk associated with use of calcium channel blockers was restricted to men (RR 1.18, CI 1.01–1.15) ($P_{interaction}$ =0.006). Similarly, when analyzed by residential ambient UVR exposure, users of agents of the RAS showed increased melanoma risk in the regions with the highest (RR 1.09, CI 1.02–1.16) and medium ambient UVR exposure (RR 1.42, CI 1.15–1.75) ($P_{interaction}$=0.023). Secondly, analyses comparing use of antihypertensive drug types (≥2 prescriptions) with non-use (≤1 prescription), exclusively or in combination with other antihypertensive drugs, showed elevated risk for exclusive users of diuretics (RR 1.14, CI 1.01–1.28), but not for use in combination with other antihypertensive drugs. For calcium channel blockers, elevated risk was found for combined use only (RR 1.12 , CI 1.04–1.20), while for agents of the RAS an elevated risk was found for combined (RR 1.07, CI 1.00–1.15) and exclusive users (RR 1.14, CI 1.05–1.23). Thirdly, the active comparator analysis, comparing use of all other cardiovascular drugs with use of antihypertensive drug types, showed elevated melanoma risk for users of diuretics (RR 1,07, CI 1.00–1.15) and for calcium channel blockers (RR 1.10, CI 1.02–1.17). Sex-specific analyses revealed the associations to be restricted to men ($P_{interaction}$=0.28 and 0.002, respectively). An elevated risk was also found for men among users of RAS agents (RR 1.09, CI 1.00–1.19) ($P_{interaction}$=0.081).

When stratified by body site, use of diuretics was associated with a slightly increased risk of melanoma at the trunk and lower limbs, and use of calcium channel blockers was associated with an increased risk at all body sites except the head/neck, though the association was only significant for trunk tumors for both drug user groups (RR 1.13, 1.03–1.24) ($P_{heterogeneity}$ = 0.45 and 0.43, respectively). Users of agents of the RAS were associated with melanoma on the upper limbs (RR 1.22, CI 1.05–1.41) and head/neck sites (RR 1.29, CI 1.11–1.49) ($P_{heterogeneity}$ = 0.045). Users of agents of the

RAS showed a 14% increased risk of SSM (RR 1.14, CI 1.06–1.23) (P$_{heterogeneity}$ = 0.45), while users of calcium channel blockers showed a 16% increased risk of other melanoma subtypes (RR 1.16, CI 1.03–1.30) (P$_{heterogeneity}$ = 0.11). Lastly, elevated risk for local stage disease was found in users of diuretics (RR 1.07, CI 1.00–1.15), calcium channel blockers (RR 1.08, CI 1.01–1.16) and agents of the RAS (RR 1.10, CI 1.03–1.17), in addition, the risk of unspecified stage melanoma was elevated in users of calcium channel blockers (RR 1.33, CI 1.07–1.66). Additional analyses of thiazide diuretics showed no associations with melanoma, including when using an active comparator design. For cumulative dose (DDD) of antihypertensive drugs, no evidence of a dose-response relationship was found across the cumulative dose quartiles for any of the drug types.

5. Discussion

This thesis describes the work on three scientific papers as part of a nested pharmacoepidemiological case-control study, investigating melanoma risk related to the intake of three classes of pharmaceutical drugs. The integrity of such studies are dependent on several principal considerations related to choice of data sources, study design, drug exposure assessment and adjustment for bias.[68] The inherent methodological strengths and weaknesses of this study will be discussed, first in terms of data sources, as well as the design and methods employed. Then the results are discussed in the context of Hills considerations as they may apply to modern pharmacoepidemiology, considering also the potential consequences methods might have on the interpretation.

5.1 Data sources

Although the melanoma incidence is relatively high within the Norwegian population, a large sample size is required to study its associations to a relatively rare exposure in a population (like use of particular drug types). NorPD has registered detailed data on drug use from 2004. To ensure a large sample of cases and information on drug-use prior to melanoma diagnoses, all melanoma cases diagnosed in Norway from 2007 to 2015, together with ten population controls per case, constituted the study sample for this study. A pharmacoepidemiological study requires that data on drug exposure is as detailed as possible, as it is typically long-lasting and variable over time.[68] Established nationwide population registries, such as the CRN and NorPD are ideal in this regard as they provide up to date and high-quality data.[143,144] Thus, a major strength of our study is that our analyses are based on a large population-based sample with detailed and well-defined data on cancer diagnoses and prospective drug use. The complete and high quality data strengthens the validity of the results from our study. However, this also imposes certain limitations.

NorPD started recording detailed information from 1 January 2004 and our study is therefore limited by this time frame. Within the sub-field of pharmacoepidemiological drug-cancer associations, any study must consider that different cancer types have variable and often long induction (cancer initiation to malignancy) and latency (cancer initiation to diagnosis) periods, which complicates the process of determining relevant drug exposure periods.[68] With an unknown latency and induction time for melanoma, the follow-up time from start of drug exposure to diagnosis/index date may have been insufficient to establish a true association. It is also worth mentioning that a date of diagnosis does not necessarily reflect the time of cancer initiation, as it depends on when the patient visited and was diagnosed by a doctor. The impact of primary non-adherence to prescription drugs by patients [152], is limited by the fact that only information on drugs actually dispensed from pharmacies to patients is recorded in the NorPD. This is more indicative of use than databases that only record all drugs prescribed by physicians alone.[153] An additional strength of this large study population is the degree of analytical resolution possible. It allows us to differentiate between sex, age groups, geographical regions, melanoma subtypes, tumor sites and stage of disease.

5.2 Methodological considerations

Several methodological aspects need to be considered when interpreting the findings of this study. This includes strengths and weaknesses regarding the choice of design, statistical methods and the internal validity with respect to bias. While the nationwide population of Norway might ideally be used as a study population in a cohort study, it would provide more data than that which would be practical for analyses. A nested case-control design was therefore chosen due to a principle of data minimization. It allows the study to include all the available melanoma cases in Norway within the relevant study period without using the entire country as a cohort, which would result in millions of observations. This design renders the study inherently prospective, in that exposure is measured before the melanoma diagnosis, which means that it produces similar estimates to a "cohort design". While the register-based nature of the study precludes certain types of bias common to case-control designs, the internal validity of the study may still be prone to selection bias, information bias, and confounding.

5.2.1 Validity

An important methodological aspect to consider is validity, the degree to which the inferences drawn from a study are warranted when account is taken of the study methods and the characteristics of the study participants.[154] The internal validity of a study is the degree to which a study is free from bias or systematic error, which is typically divided into selection bias, information bias and confounding. The external validity is the degree to which results may apply, be relevant, or be generalized to populations or groups outside of the study population, and depends on the internal validity.[154]

Selection bias

Selection bias is bias in the estimated association of effect of an exposure on an outcome that arises from the procedures to select individuals into the study or the analysis.[154] In the case of this study, all melanoma cases and controls in a given time frame were included. However, controls without information about county of residence (the proxy for UVR exposure) in the NorPD were excluded (3%). These 3% consist by definition of those not registered in the NorPD and thus did not receive any drug prescriptions. They could for example represent exceptionally healthy persons or long-term inpatients. This exclusion may have introduced a degree of selection bias. However, due to the small size of this proportion, we expect the impact on the results to be minimal.

Information bias

Information bias occurs when exposures, covariates or outcomes are measured with error or misclassification, resulting in different quality of information between comparison groups.[154] Measurement errors are differential if they depend on any other variables and non-differential if independent of any other variables (between

covariates). In the present study, misclassification of variables is possible, but unlikely with respect to the main variable. However, as all individuals were classified in the same manner into outcome and exposure categories, any such misclassification should be non-differential.

Recall bias (differences in accuracy or completeness of recall to memory of past events or experiences between cases and controls[154]) is common in case-control studies, however, this study is based on data obtained entirely from nationwide high quality health registries, with mandatory reporting and high completeness, which precludes recall bias.

In the present study, all data related to melanoma as an outcome was obtained for all cases diagnosed in the given age span and time frame from the CRN, a validated and high quality nationwide register.[12,143] Therefore, outcome misclassification is not likely. The sampling of controls was done with replacements, as previously explained. A control could therefore become a case and would then be analyzed as both case and control at different time points and as different observations due to their unique risk set-id. However, this would not introduce any outcome misclassification.

Similarly, data on drug use as the main exposure as well as other covariates of interest were all obtained from nationwide health registries with accurate reporting and a high degree of coverage[143,144] and categorized the same for cases and controls. Despite these qualities, data obtained directly from prescription registries cannot be relied upon to completely reflect real-life individual exposure. While the agreement between self-reported use and prescription registries is often good, it varies depending on drug class and medical histories.[155] Self-reported use can also be lower than registered data as some prescriptions dispensed are not used in full, or indeed may not be remembered and/or reported by the user.[156] In a validation study investigating drug use from the NorPD during pregnancy and risk of hypertension in newborns, including longer (+90 days) pre-pregnancy exposure windows lead to lower specificity and an underestimation of risk when compared to self-reported use.[157]

In addition, we were unable to account for drug use in any person prior to the establishment of the NorPD (1 January 2004). This could have led to a misclassification of a person's true drug exposure, both in terms of number of prescriptions and cumulative dose. A way to control for this could be to remove all persons with drug use in 2004, though this demanded the removal of too many study participants. According to a recent sensitivity analysis, this lead to a considerable loss of power, though retained the same trends.[158] Thus, misclassification and underestimation of drug use may be likely. The implementation of "lag-time" is also important to avoid reverse-causation bias. An attempt to control for this type of bias was made by excluding prescriptions received up to one year prior to (and after) the diagnosis/index date.

The presumed duration of use is the principal aspect that we considered when defining both the number of prescriptions and cumulative dose (DDDs). When categorizing number of drug prescriptions we combined non-use and minimal use in the same category (0–1 prescription). The Norwegian reimbursement regulation states that pharmacies can dispense drugs sufficient for 3 months of use per dispensing. Thus, a single prescription reflects non-use or very limited use. Also worth mentioning is that

long-term drug therapy regimens may consist of several different drugs, sometimes taken in combination, and with varying lengths and degrees of exposure. Additionally, the number and combinations of these drugs may change over time.[91,92] Aside from small user groups, this makes it difficult to narrow the risk of melanoma down to one specific drug, as an increased risk of melanoma may be due to the cumulative effect of the entire drug therapy regimen. This challenge was particularly prevalent for immunosuppressant and cardiovascular antihypertensive drug user groups.

Confounding

In the study comprising this thesis, potential confounding factors should be taken into account as they may influence the association between drug use and melanoma risk. A confounder is a variable that is associated with the disease in question (not as an effect of the disease, but as a cause or a proxy for a cause), and with the exposure, but it is not itself an effect of the exposure.[159] Such confounding variables may create spurious associations or mask a real association between exposure and outcome.

Individual information regarding UVR exposure, a major environmental risk factor for melanoma [13,17], was lacking in our study. UVR could chemically interact with certain drugs with photosensitizing potential (Table 2), which could have further exacerbated skin damage and increased skin cancer risk. In such cases however, UVR constitutes a part of the pathway towards melanoma development and is therefore not considered a confounder. However, the effect of UVR on melanoma risk may also be affected through indications for drug use, thus making it an important confounding factor.[109,123,124] The region of residence, previously shown to be a suitable surrogate for UVR exposure [23], was thus used as a proxy to account for the influence of ambient UVR exposure for each person, which decreases from southern to northern Norway.

The study has no information regarding the underlying indications for drug use, although the primary indications for which drugs are prescribed can be inferred from drug databases.[160] The lack of such information could potentially have introduced confounding by indication. This type of confounding would occur if the underlying condition for which a particular drug is prescribed also influences the risk of melanoma. The effects of depression and mood disorders have been linked to a positive effect on several cancer promoting pathways[161,162], and states of stress, experiences of traumatic events and depression have all been associated with an increased risk of melanoma.[78] However, depression may also cause reduced social activity and may hence change the length and patterns of UVR exposure.[163] Depression would in this case be a mediator, predisposing antidepressant users at a different risk of melanoma through altered UVR exposure. Also, if we are to consider antidepressant use an indicator for conditions related to depression, one must also account for the fact that long-term use of antidepressants reduces and mitigates the effects of depression by normalizing the neurological pathway alterations that could contribute to carcinogenesis.[162]

Likewise, certain autoimmune skin disorders (e.g. psoriasis) can, in addition to immunosuppressive drugs, be treated with high-dose phototherapy which may increase the risk of melanoma.[164] The varying indications and their degrees of severity may also be associated with other relevant factors (e.g. obesity, socioeconomic factors, hormone use, and alcohol consumption), neither of which we had information about. An active comparator design was used in paper III to help minimize the impact

of confounding by indication, but apart from confining the risk to men in users of RAS agents, obtained similar results to that of the full case-control analysis.

Moreover, as most people in Norway reside in the southern and eastern regions of the country, we cannot discount the possibility that intra-regional differences regarding prescription practices and access to healthcare (diagnostic intensity) have influenced both drug use and melanoma risk.[70] This is mitigated, however, by our adjustment for region of residence, and for other drug use as a proxy for healthcare usage.

Finally, to further deal with potential confounding, we utilized the available literature to discuss whether both unobserved and observed factors have influenced the association between drug use and melanoma risk in paper I-III, and to evaluate the causality of the observations.

5.2.2 Statistical methods

Conditional logistic regression was used in papers I–III, to estimate the association between drug use and melanoma, while taking information on potential confounding factors into account. The effect measure depends however, on how the controls are selected. In the present study, the controls were selected longitudinally throughout the course of the study period, using risk-set sampling. The matching on age as a time-dependent variable means that the controls estimate the exposure odds in the study base, represented by person-time at risk.[149] Therefore, the effect estimates in the papers of this thesis were reported as RRs (rate ratios). To evaluate the precision of the estimates, 95% CIs were calculated.

Interaction effects

A statistical interaction is present when the effect of an exposure, compared with a unexposed reference group, depends on the presence of one or more covariates.[165] As described above (section 3.4) we tested for statistical interaction between the number of drug prescriptions and sex, age (at diagnosis/index date), residential ambient UVR exposure (three level variable) and parity (women only) on melanoma risk.

In paper I, use of antidepressants (≥8 prescriptions) was associated with a reduced risk of melanoma in men and in persons >70 years at diagnosis/index date. In paper II, use of corticosteroids (≥8 prescriptions) was associated with a reduced risk of melanoma in men. In paper III, the elevated risk of melanoma associated with calcium channel blockers (≥2 prescriptions) was restricted to men, and the elevated risk of melanoma associated with agents of the RAS (≥2 prescriptions) was restricted to the regions with high and medium ambient UVR exposure. Real interactions may go undetected however, because the test for interaction lacks power. [166]

Disease subtype heterogeneity

As described above (section 3.4), we also performed tests for heterogeneity to assess whether exposure-disease associations differed by melanoma sites, histological subtypes and clinical stage at diagnosis. In paper I, we found a reduced risk among antidepressant users for the trunk, upper and lower limb sites, all histological subtypes,

as well as for local disease. However, it is uncertain whether any of these disease-exposure associations differed significantly as tests for heterogeneity were not performed for any disease subtypes. In paper II, we found an increased risk among immunosuppressant users for the head/neck, trunk and upper limb sites, all histological subtypes and for local and regional stage of disease. However, no significant heterogeneity between any disease subtypes was found. This means we cannot be certain whether users of immunomodulating drugs are subject to different risks of melanoma of any particular body site, histological subtype or clinical severity. In paper III, an increased risk was found for trunk sites and local disease among users of diuretics. An increased risk was found for trunk sites, other histological subtypes, and local and unspecified stage for users of calcium channel blockers. An increased risk was also found for upper limbs and head/neck sites, SSM, and local disease among users of RAS agents. However, tests for heterogeneity were only significant for users of RAS agents by body site, confining the increased risk to head/neck and upper limb melanomas.

5.3 Discussion of the main findings

In 1965, the epidemiologist Bradford Hill proposed nine considerations for guiding the separation of causation from mere association. These included strength of association, consistency (replication) of results, specificity, temporality, biological gradient, plausibility, coherence, experimental findings and analogy.[167] While these considerations left a very influential legacy in the field of epidemiology, they do not serve as a checklist for establishing whether an association is causal or not. Nor does any consideration alone serve as evidence of causality between an effect and an outcome.[167-169] The methods and criteria for guiding the separation of causality from association have advanced considerably since Hill proposed his considerations, and their applicability and limits have frequently been brought into question, with more modernized approaches being advocated.[168-170] While recognizing the limitations of Hill's considerations, the findings of the study featured in this thesis are discussed within its framework, as much as they may apply to a modern pharmacoepidemiological study.

5.3.1 Strength (effect size)

Hill's first consideration states that the strength of an association shares a positive relationship with causality.[167] This means that weak associations are less indicative of causality than stronger ones, and are more likely to be the result of bias. However, modern interpretations state that strength is not just a measure of the magnitude of the association, as certain risk factors may produce a small yet statistically strong association, which itself can support a causal relationship.[168] Strengths of associations are generally not considered important for causal inference, as small and large effects can be equally plausible. In fact, it is suggested that small effects may be more indicative of causality than larger effects, the latter of which could also result from error or bias, and small effects may still be relevant to clinicians and for public health.[169] However, the effect size obtained also depends upon the statistical methods used. Even when strong and statistically significant associations are obtained, they may not

always be appropriate based on the methods used or indeed biologically meaningful.[168] Neither does this mean that a lack of a significant association precludes the existence of a causal relationship between an exposure and outcome.

In our study, we found statistically significant differences in melanoma risk among users of prescribed drugs compared to non-users. However, the direction and the magnitude of the associations differed. For users of antidepressants we observed a decreased melanoma risk. The associations were statistically significant, yet of moderate magnitude, with a 19% decreased risk for antidepressant use overall (RR 0.81, CI 0.75–0.87), and 18% and 23% for SSRIs (RR 0.82, CI 0.73–0.93) and mixed use (RR 0.77, CI 0.69–0.86), respectively. On the other hand, users of immunosuppressants were generally at considerably higher risk of melanoma than non-users. A 50% increased risk of melanoma was found for users of immunosuppressants overall (RR 1.50, CI 1.27–1.77). This was especially amplified when examining users of immunosuppressants typically prescribed to OTRs, in which the risk was more than doubled. Similarly, elevated melanoma risk was found for selected antihypertensive drugs, although with weak effect estimates in general. The associations persisted when variables that potentially could influence the drug-melanoma association were taken into account. However, not all such variables could be controlled for. Use of nationwide registry data and a large number melanoma cases gives the results increased statistical power, although we cannot rule out that the results of sub-analyses could suffer from low power due to the few number of users in these groups. The effect estimates have also been evaluated according to existing literature.

While these associations were significant, and was particularly strong for users of OTR drugs, they are not indicative of causality, nor causal relationships of different strengths. Other drug-melanoma relationships may yet exist and those we found may stem from factors other than drug agents.

5.3.2 Consistency and coherence

Hill's consideration of consistency stresses the importance of corroborating findings within a study, but foremost whether other studies from a variety of disciplines with variable study populations and methods support our findings.[167] According to Hill, this consideration is important as, due to limitations in materials and methods, no single study can be relied upon to infer causation.[167] Consistency is arguably one of the most important contributing considerations when it comes to substantiating a possible causal relationship. This also provides a basis for Hill's seventh consideration, coherence, which states that all current knowledge regarding the association between the exposure and outcome should support the findings of the present study.[167] Coherence is often difficult to operationalize though due to its rather vague definition and applicability, and is therefore discussed here in conjunction with consistency.[169] We have discussed our findings according to supporting evidence from epidemiological, as well as pre-clinical drug studies.

For antidepressant drugs, *in vitro* and *in vivo* drug studies have reported conflicting results, although the prevailing indication is that drug exposure exerts an inhibitory effect on melanoma development.[80-86] While epidemiological studies with a focus on melanoma risk are lacking, findings on the associations between antidepressant drug

use and other cancer types are reported.[171] However, recent studies find little evidence of an increased risk of cancer in antidepressant users, which is even reduced for some cancer types.[85,87,88] The results of our study are in concurrence with both pre-clinical studies in how they relate to the inhibition of melanoma growth and progression, as well as with more recent epidemiological studies on the risk of other cancer types, particularly with regard to the use of SSRIs. However, in our study, it is arguable whether the negative association result from antidepressant use itself or from low exposure to UVR. Sub-analyses (by sex, age, residential ambient UVR exposure) also pointed in the direction of low UVR exposure as a potential explanation, although potential biological effects of drug agents cannot be ruled out.

The results of *in vitro* and *in vivo* studies investigating associations between certain immunosuppressant drugs and melanoma have reported that exposure triggers a growth inhibition of melanoma cells.[172-174] Epidemiological studies investigating this relationship however, overwhelmingly show that users of immunosuppressive drug regimens, particularly those for OTRs, exhibit an increased risk of melanoma as well as KC.[66,94,96,99,101,103-108,175,176] This is in line with the results of our study, which found that immunosuppressant users are at higher risk of melanoma, including methotrexate users and particularly users of drugs for OTRs. However, no association with melanoma was found for "Other drugs with immunosuppressant actions", and we were not able to clarify the indeterminate findings in previous studies for these drugs [95,177], partially due to small user groups. We did not find heterogeneity in body site, histological subtype or clinical stage for immunomodulating drug users. This, combined with an increased risk of all histological subtypes for immunosuppressant users, could indicate that immunosuppressant drugs play a role in melanoma development through a common mechanism of effect.

Epidemiological studies examining the associations between use of corticosteroids and skin cancers have found that users of glucocorticoids exhibit an increased risk of KC and cutaneous T cell lymphoma[116-119], though the association with melanoma seems not to have been examined. Apart from a reduced risk in men, and in persons residing in the medium ambient UVR region, no associations were found between corticosteroid drug use and melanoma in this study. *In vitro* studies of corticosteroids however, have primarily found that glucocorticoid exposure exerts an inhibitory effect on the growth of melanoma.[113-115]

For antihypertensive drugs, the available *in vitro* and *in vivo* drug studies indicate that many of these drugs may have negative effects on the growth of melanoma cells, particularly calcium channel blockers and agents of the RAS.[120] Epidemiological findings on the subject generally indicate that cardiovascular drug use overall does not represent a risk factor for melanoma development or progression.[120] However, the use of several antihypertensive drug types have been associated with an increased risk of skin cancer, including melanoma.[65,120,129] Our results for specific antihypertensive drug groups corroborate several contemporary epidemiological findings, including an increased risk of melanoma for users of diuretics and agents of the RAS (possibly due to photosensitizing effects).[65,120,129] Our study also found an increased risk of melanoma in users of calcium channel blockers (in men). This is in concurrence with several epidemiological findings, which show that use of calcium channel blockers is associated with an increased risk of cancer, including melanoma.[65,120,129]. However, the significance and strength of these associations vary across the literature (due to differences in sample size and statistical power). Other studies however, suggest that calcium channel blockers may even have a future role as an adjunct to cancer

therapy.[120] To sum up, the observed drug-melanoma relationships in the papers of this thesis are largely supported by both pre-clinical and epidemiological findings.

5.3.3 Specificity

Hill's third consideration is specificity, which stipulates that causality is more likely if we can ascertain that an exposure is solely responsible for one outcome (disease).[167] In nature, and thus the field of modern epidemiological study, this is an unlikely situation, as it would require complete knowledge and control over all factors that could potentially influence the outcome, and account for them in analyses. Its validity as a consideration for inferring causality is therefore largely undermined by the reality that most exposure-related diseases are to different extents caused by a variety of competing risk factors.[167-169] Melanoma risk depends on sex, age and various factors related to individual susceptibility and heredity, and exposure to UVR, the major environmental risk factor. Other exposures and lifestyle-related factors are also suggested to influence the risk.[49,50,57,58,60] However, we did not have complete knowledge of this and thus could not control for all these factors.

It is particularly prudent to consider the influence drugs might have for a disease as etiologically heterogeneous as melanoma, both in terms of anatomic site, histological subtype and genetic profile. Even though UVR exposure accounts for a large proportion of melanoma cases, it does not impact melanoma development in a unified manner.[3] While certain users of the prescribed drugs in focus exhibit significant associations with melanoma risk, the lack of adjustment for potentially confounding factors like socioeconomic status and indications for drug use means that we cannot be certain that any drug type alone specifically causes melanoma.

5.3.4 Temporality

Hill's fourth consideration states that an exposure has to precede the outcome by a time period within which it is capable of affecting the initiation and development of a disease.[167] The importance of this consideration is undisputed in epidemiological research, and is essential when establishing causality. However, temporality may be difficult to document and establish for a variety of conditions with long and unclear latency periods, such as melanoma.[169] In our study, drug exposure was obtained from 2004–2015, and included melanoma cases diagnosed from 2007–2015. Prescriptions filled after and a year prior to date of diagnosis/index date were disregarded for any case or control for the analyses in this thesis. Thus, we were able to assess drug use exposure for a minimum of three years and a maximum of 11 years prior to the diagnosis. However, it is uncertain whether the limited time of exposure to diagnosis/index date, was enough to influence melanoma development as this process has been proposed to take up to 10 years.[178] Thus, a limitation of this study is the potentially short latency time between drug exposure and melanoma diagnosis.

In paper I, separate analyses by duration of use showed an association in particular for long-term users (>5 years), indicating a need of exposure for a certain time-period. We must also consider that no individual data exists on prescription drug use before 1 January 2004, and that the study cannot account for individual drug use before this

date. This unmeasured potential exposure before the study period could lead to an overestimation of any measured drug effect. However, as our understanding of melanoma initiation and progression increases through advances in the use of new techniques such as biomarkers and chemical exposure monitoring and analysis, the value of temporality will increase as a measure of causality inference.[168]

5.3.5 Biological gradient (dose-response)

Hill also emphasized that causality is more probable if there is a dose-response relationship between an exposure and the outcome.[167] However, how to evaluate and interpret that gradient is challenging. Dose-response relationship are not necessarily strictly monotonic, but can be non-linear or threshold-dependent and can vary considerably between studies.[168,169] We did not observe a firm dose-response relationship for use of antidepressants, immunomodulating, or antihypertensive drugs. While the risk of melanoma decreased along with the number of prescriptions of antidepressants (overall, SSRIs and mixed use), and increased for increasing number of immunosuppressive drug prescriptions (OTR drugs and methotrexate), the effects for users of 2–7 prescriptions were not significant. This is likely the result of a low number of users in this category. For this reason, in the analyses of antihypertensive drugs, the prescription number categories 2–7 and ≥8 were collapsed into "users" (≥2 prescriptions).

Moreover, when estimating melanoma risk by cumulative drug exposure (DDD), users of the lowest dose (1–91 DDD for antidepressants and 1–365 DDD for immunomodulating drugs) constituted the reference category in papers I–II. This was done in an attempt to account for confounding by indication, which could provide more reliable estimates of potential dose-response relationships for cumulative drug exposure. Non-use (0 DDD) was chosen as a more orthodox reference level for antihypertensive drugs as this level provides no exposure. For antidepressants, non-users exhibited a significantly increased risk of melanoma, while for immunosuppressants there were no significant differences between the lowest dose level and the cumulative dose categories. For antihypertensive drugs, the increased melanoma risk did not change significantly between the cumulative dose levels. Such monotonic dose-response curves however, would likely be over-simplified representations of most true causal relationships.[168] More complex, non-linear dose response relationships have in fact become the norm rather than the exception, as Hill predicted.[167,168] The obtained results indicate that if the risk of melanoma is indeed reduced by the use of antidepressants or increased by use of immunosuppressants and selected antihypertensive drugs, it seems to be threshold dependent and does not appear affected by intensity or duration of drug exposure.[168] However, we cannot rule out that a different dose-response relationship could exist for these drug types as certain user categories suffer from low power.

5.3.6 Plausibility and experiment

Hill also stated that biologically plausible mechanisms should exist between the exposure and outcome to infer causality. However, biological plausibility depends largely on the current state of knowledge regarding the mechanisms and etiology of

the disease being studied, and was not necessarily a requirement by Hill.[167] Due to varied disease etiologies, demonstrating definitive biological plausibility for inferring causality can also be challenging. However, there is a base of immunological studies and pre-clinical drug studies which elucidate the mechanistic background for drug effects and melanoma development, making this a more valid consideration for corroborating the possible causality of observed associations. Hill also emphasized the utilization of findings from experimental manipulation studies, in which interventions or cessation of a particular exposure affects the subsequent risk of a disease. While this consideration is still considered important [169], its applicability and validity is still limited by the current state of knowledge, study design and disease being studied. As previously mentioned, we know that cancers, including melanoma, are likely the result of several competing and interacting risk factors. The long and unknown latency time for melanoma also has to be considered, as initial development of melanoma may have been triggered by exposure received several years ago. Intervention or cessation of drug use may therefore not affect melanoma risk within the time frame of a study. It is therefore discussed here in conjunction with biological plausibility.

Experimental studies on the effects of antidepressants indicate that such drugs may exert an inhibitory effect on the growth of melanoma cells. The drug agents are thought to inhibit and/or arrest the malignant cell cycle in both *in vitro* cancer cells and *in vivo* tumors, and are demonstrated to induce apoptosis.[84-86] However, the receptors targeted by antidepressants to induce this anti-carcinogenic effect remain unknown. Additional studies are required to elucidate the molecular pathways by which antidepressant agents exert their effect, particularly with regard to newer drug classes like the SSRIs.

It is also suggested that antidepressants can modulate immune function and thus act as mediators of cancer suppression and tumor progression.[85] Lymphocytes are known to express several neurotransmitter transporters, which may be affected by exposure to antidepressants. Most studies report a stimulatory effect of such drugs on immune function, which is relevant as melanoma development is partly an immune-mediated process.[93] Animal studies on the effects of antidepressants report increased T-cell and splenocyte proliferation and Natural Killer cell activity, as well as alterations in cytokine levels, including interleukin-6, a mediator of the of the antitumor response against melanoma.[85] Such studies would suggest that a link does exists between the immune system and the changes in tumor growth caused by antidepressant exposure.[80,81]

Melanoma in users of immunomodulating drugs is theorized to be the result of reduced immune surveillance of oncogenic changes due to the chronic immune suppression caused by the drug.[179] There is also a firm body of evidence which establishes immunosuppression as a potent risk factor for the development of melanoma in a range of immunocompromised patient populations.[179] However, it is unclear whether immunosuppressants directly cause melanoma, or if they indirectly do so by creating a preferable environment for its development.[179] Melanoma is an immunogenic tumor that spreads through the lymphatic system, relying on factors that trigger an immunosuppression of the tumor microenvironment, which allows it to evade the body's tumor surveillance system.[180,181] The immunological modulation by which melanoma development is triggered however, is still poorly understood, as is the molecular microenvironment of melanoma. Thus, melanoma-induced immunosuppression could be amplified by exposure to immunomodulating drugs.[179,181] However, determining the melanoma risk caused by use of individual

immunomodulating drugs is difficult due to multidrug regimens and confounding factors due to the indications for their use.

The propensity of certain immunosuppressants to exacerbate UVR–induced DNA damage is also theorized to be potentially responsible for an elevated risk of melanoma. This raises the question of the extent of synergy shared between immunosuppression and UVR exposure. Cumulative UVR and fair skin have been associated with an increased risk of KC in OTRs[182], though studies of OTRs in areas with less ambient UVR show no higher risk of skin cancer.[183,184] We found an increased risk of melanoma for immunosuppressant users in the regions with medium and highest ambient UVR, which lends credence to the potential effect of photosensitization.[20] Though one must consider that a lack of individual UVR exposure metrics and low power due to small groups, may explain why a similar association was not observed in the low ambient UVR region. Moreover, the associations with body site were generally strong, and a particular disparity was observed for melanoma of the head/neck in users of immunosuppressants, where the risk was increased two-fold. The head/neck location is subjected to chronic low-intensity UVR exposure, while the trunk typically receives intermittent high-intensity UVR exposure. Thus, our results could point towards a potential interaction between UVR exposure and immunosuppressant drugs with photosensitizing potential.[109,124,185]

Experimental evidence shows that the progression of severe skin cancer may be slowed by cessation or reduction of particular immunosuppressant drugs in OTRs. This includes lowering dosage levels, removing one agent (typically azathioprine), or switching one immunosuppressant agent with one that is less oncogenically permissive. This includes switching from calcineurin inhibitors to specific immunosuppressants like the mTOR inhibitors sirolimus and everolimus. *In vivo* studies also suggest that certain immunosuppressants, including mTOR inhibitors can inhibit melanoma tumor growth while protecting the transplanted tissue.[186-188]

In vitro studies of corticosteroids have found that some of these drugs exert an inhibitory effect on melanoma growth, primarily through suppression of angiogenesis and cellular growth.[113-115] Analyses of biopsy samples provide biological underpinnings for such a relationship, which show that human melanoma cells have large quantities of glucocorticoid receptors similar to those found in normal tissue, and may thus be of therapeutic value.[113]

Continued tumor growth requires a steady and ample blood supply, which has lent credence to the hypothesis that drugs that decrease vascular blood flow could inhibit cancer progression.[128] Moreover, the molecular targets of several cardiovascular drug agents are not limited to the cardiovascular system itself, but also include the cells of other systems, such as immune cells.[189] In theory, this could affect the growth and progression of immunogenic cancers like melanoma.[190] The potential role of beta-blocking agents has biological underpinnings, as melanoma cells express both β1- and β2-adrenoreceptors. When activated, these receptors promote angiogenesis and tumor growth by stimulating the production of vascular endothelial growth factor and interleukin-6 and -8.[191] Inhibition of these receptors by beta blocking agents thus has the potential to inhibit pro-tumorigenic effects with regards to melanoma growth.[120] However, no associations were found between the use of beta blocking drugs and melanoma risk in the present study. The RAS has roles in both oncogenesis and tumor suppression functions and the influence of angiotensin II in this regard supports the hypothesis that ACEIs and ARBs may inhibit melanoma growth.[192] L- and T-type

calcium channels are targets for calcium channel blockers and are found to be present and functional in melanoma cells, but not in normal melanocytes. Contrary to our results, treatment with L-type calcium channel blockers has been shown to reduce melanoma cell viability and has inhibited the proliferation of uveal melanoma cell lines. Use of T-type blockers also indicate an inhibitory effect on melanoma growth.[120] Thus our finding of increased risk with use of calcium channel blockers is not supported by experimental evidence.

Most antihypertensive drugs have also reported adverse photosensitizing effects among users[124] that might contribute to elevated melanoma risk.[65,120,129] Such a concern relates primarily to the use of diuretics and particularly so with regards to thiazide-like diuretics, which in epidemiological studies have been associated with increased melanoma risk[131,193,194], though not in all populations.[133] While additional analyses of thiazide diuretics revealed no significant associations with melanoma in this study, drug-induced photosensitization may be a contributing factor to the increased risk of melanoma found in users of diuretics, calcium channel blockers and RAS agents.

The findings in current literature provide a mechanistic basis for the effects of drug agents on melanoma and support biologically plausible explanations for the associations found in our study, for select groups of antidepressant, immunosuppressant and antihypertensive drug users. For immunosuppressant drugs in particular, but also for selected antihypertensive drugs, the results also point towards adverse photosensitizing side effects as a potential explanation for our findings. On the other hand, we cannot rule out that other mechanistic drug effects may influence our results and that varying and often severe individual indications for use could contribute to the observed changes in melanoma risk.

5.3.7 Analogy

Hills final consideration concerns corroborating your findings between a similar exposure and/or outcome.[167] In this case that means that the causality of the association found between the drug types in focus and melanoma could be strengthened by findings in other studies, even those concerning similar drugs and/or cancers. If the same drug type was associated with KC or indeed other malignancies, the case for inferring a causal relationship between the drug and melanoma may be strengthened. While inferring causality through analogy may be valid in certain circumstances, it is a fairly weak consideration, particularly for pharmacoepidemiological associations with cancer, in which many significant but non-valid relationships may exist with a variety of drugs due to unmeasured confounding.[169] This consideration has largely been taken into account however, as corroborating findings concerning the three drug types in focus are discussed above in accordance with several of the previous considerations.

While another drug type with similar effects could represent the analogous causative agent, a biological state analogous to the indication for which the drug is given or one imposed by the drug in question may also serve as an analogue for being a user of the drug itself. However, this would imply that it is not the drug itself, but the biological states caused by the drug or for which it is given, which causes melanoma. While status as independent risk factors is not well founded, states of stress, experiences of traumatic events and depression have all been associated with an increased risk of

melanoma.[78] Similarly, different immunocompromised patient populations are also subject to an increased risk of melanoma.[179] While this would conflict with the drug-melanoma associations observed in this thesis, the potential lack of UVR exposure as the main environmental risk factor, may outweigh the biological states indicated for or imposed by the drugs in question.

5.4 Conclusions

- In paper I, the principal findings concerned a decreased risk of melanoma exhibited by persons with long term and/or high-intensity exposure to antidepressants overall, SSRIs and other antidepressants. This could indicate that exposure to antidepressant drugs prevents the development of melanoma through cancer-inhibiting actions induced by the drugs. Alternatively, the depressive conditions for which these drugs are prescribed may predispose these patients to reduced UVR exposure and thus a reduced melanoma risk. This remains a distinct possibility as we lack information concerning UVR exposure. Thus, we cannot recommend the use of antidepressant drugs as a prophylactic measure against melanoma. To investigate this further, studies with individual information about UVR exposure and indication for drug use are required.

- In paper II, we found increased risk of melanoma in persons with high-intensity immunosuppressant drug exposure. This association was also found for users of methotrexate, but was particularly strong in users of drugs given to OTRs. General immunosuppression and possible adverse photosensitizing side effects from UVR exposure may explain these findings. Corticosteroid exposure did not seem to increase the risk of melanoma. Our findings suggest that users of immunosuppressant drug types, particularly OTRs, comprise a notable risk group, and so should in addition to regular skin check-ups, pursue a more cautious approach to UVR exposure. No recommendations can be made for users of systemic corticosteroid drugs, as the relevant findings in this paper were inconclusive.

- In paper III, an increased risk of melanoma was found for users of the antihypertensive drugs; diuretics, calcium channel blockers and agents of the RAS. Overall, the results suggest that users of selected types of these drugs are subject to an increased risk of melanoma, which may be due to a synergistic effect from UVR-induced photosensitization. However, small effect sizes and lack of dose-response associations suggest no causal association with drugs. Regardless, guidance towards promoting more cautious sun exposure habits may be considered for users of these drugs due to their photosensitizing potential. For other types of antihypertensive drugs, no association with melanoma risk was observed, giving no indication of any recommendations for users.

5.5 Public health implications

Melanoma incidence and mortality are increasing in European countries and has remained the most rapidly increasing cancer type in Norway for the past two decades.[9,10,12,14] While the risk of melanoma depends on host factors, the primary risk factor remains exposure to UVR, from the sun and tanning devices.[13] The number of users of prescription drugs however, has steadily increased alongside melanoma rates, and nationwide pharmacoepidemiological findings regarding the associations between drug use and melanoma are lacking. Having investigated the association between exposures to three major drug classes and melanoma, the study featured in this thesis has made several interesting findings with potential implications for public health.

It is our hope that the findings of this study may be used to help improve the targeted prevention of melanoma through the identification of high-risk individuals. This especially concerns users of immunosuppressant and antihypertensive drugs, as well as future health campaigns and surveillance programs for patients with a risk profile predisposing for melanoma development. The results can help facilitate more informed and thus safer use of such drugs.

5.6 Future research directions

As with many cancers, melanoma is a heterogeneous disease, both in terms of site, histological subtype and genetic diversity, and it is unlikely that any drug type or other type of exposure could act as a universal carcinogen.[3,68] The clinical differences most likely reflect different etiologies, which are again influenced by several competing risk factors.[3] The lack of information on individual UVR exposure is a weakness in our study. It remains the major environmental risk factor, but the risk of melanoma depends on an interaction between the pattern and amount of UVR received and the exposed sites and host factors. To elucidate this matter further, additional prospective studies with individual data regarding UVR exposure, host factors, drug use and indication for drug use are required. When combined with prospective data on personal exposure metrics, such as UVR, reliable nationwide registers like the CRN and NorPD will prove valuable assets in future studies. The value of this data will only increase as it accumulates over time. This will help us to account for the unknown latency time of melanoma by allowing for longer follow-up times.

The findings in pharmacoepidemiological studies can be strengthened by corroborating evidence from pre-clinical drug studies. Elucidating the molecular mechanism of efficacy for newer antidepressant classes, the molecular pathways by which melanoma is triggered as well as the immunological state of tumor microenvironments and the photosensitizing potential of drug agents are all priorities in this regard. Improved statistical methods for causal inference have also become increasingly applied in recent years.[195] This could provide prospective pharmacoepidemiological studies with a higher degree of resolution in terms of how an analysis handles several intersecting risk factors, confounders and mediators, like genetic profiles, pigmentation characteristics and individual UVR exposure metrics. This in turn could disentangle and illuminate the biological pathways by which a drug may initiate oncogenesis.[168]

The use of randomized controlled trials (RCTs) has been a vital tool for demonstrating regulatory drug safety and efficacy and has so far served as the gold standard. However, they are limited by a small study population and strict parameters, and cannot therefore guide the clinical use of the drug in question.[196] Observational studies can often make a stronger claim to external validity than RCTs because they are often more representative of the target population of interest.[195] Moreover, the long process of cancer development and the latency of any carcinogenic and antineoplastic drug effects contribute to the considerable time it takes to fully elucidate potential drug–cancer relationships. Real-world data can include clinical data gathered from a population outside the limited confines of RCTs, as well as from other completed RCTs.[196] In the UK, an initiative called "care.data" gathers patient data from general practitioners and links it with hospital records.[197] Factors related to technical readiness and concerns about data privacy issues represent substantial impediments to its widespread implementation. However, the evidence continues to support real-world data studies as important supplemental sources of information together with or in the absence of RCTs, and can make substantial contributions to regulatory decision-making related to clinical pharmaceutical drug use.[196]

6. References

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Paper 0

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Cardiovascular, antidepressant and immunosuppressive drug use in relation to risk of cutaneous melanoma: a protocol for a prospective case–control study

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ABSTRACT

Introduction The incidence of cutaneous melanoma (hereafter melanoma) has increased dramatically among fair-skinned populations worldwide. In Norway, melanoma is the most rapidly growing type of cancer, with a 47% increase among women and 57% among men in 2000–2016. Intermittent ultraviolet exposure early in life and phenotypic characteristics like a fair complexion, freckles and nevi are established risk factors, yet the aetiology of melanoma is multifactorial. Certain prescription drugs may have carcinogenic side effects on the risk of melanoma. Some cardiovascular, antidepressant and immunosuppressive drugs can influence certain biological processes that modulate photosensitivity and immunoregulation. We aim to study whether these drugs are related to melanoma risk.

Methods and analysis A population-based matched case–control study will be conducted using nation-wide registry data. Cases will consist of all first primary, histologically verified melanoma cases diagnosed between 2007 and 2015 identified in the Cancer Registry of Norway (14 000 cases). Ten melanoma-free controls per case (on date of case melanoma diagnosis) will be matched based on sex and year of birth from the National Registry of Norway. For the period 2004—2015, and by using the unique personal identification numbers assigned to all Norwegian citizens, the case–control data set will be linked to the Norwegian Prescription Database for information on drugs dispensed prior to the melanoma diagnosis, and to the Medical Birth Registry of Norway for data regarding the number of child births. Conditional logistic regression will be used to estimate associations between drug use and melanoma risk, taking potential confounding factors into account.

Ethics and dissemination The project is approved by the Regional Committee for Medical Research Ethics in Norway and by the Norwegian Data Protection Authority. The study is funded by the Southeastern Norway Regional Health Authority. Results will be published in peer-reviewed journals and disseminated further through scientific conferences, news media and relevant patient interest groups.

Strengths and limitations of this study

► Linkage between four nation-wide population-based registries through unique personal identification numbers produces comprehensive, complete and high-quality data for analysis.
► A high number of melanoma cases with information on drug use prior to the melanoma diagnosis further enhances the strength of the study.
► The latency time between drug exposure and melanoma diagnosis is uncertain and in the case of this study, it may not be sufficient to infer a relation between drug use and cancer development.
► Data pertaining to measures of residential ambient ultraviolet exposure is available, but data on recreational sun exposure, everyday sun exposure, sunburn, solarium, family history of melanoma, educational level, anthropometry and hormone use as potential confounders are lacking.

INTRODUCTION
Rationale and evidence gaps

Cutaneous melanoma (hereafter melanoma) is the most lethal form of skin cancer. During the period 2000–2016, a remarkable increase in the age-standardised incidence of melanoma has been seen in Norway, with a 57% and 47% increase among men and women, respectively, making melanoma the fastest growing malignancy in Norway.[1] Norway is ranked among the top five worldwide in age-standardised melanoma incidence rates, years of healthy life lost and mortality.[2]

Ultraviolet (UV) radiation from sun and solarium, which is classified as a human carcinogen by the International Agency for Research on Cancer (IARC),[3 4] was responsible for approximately 75.7% of all new

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melanoma cases worldwide in 2012.[5] The development of melanoma is, however, a multifactorial process, with risk also depending on individual susceptibility. These include certain phenotypic characteristics,[6] a previous melanoma diagnosis,[7] family history of melanoma,[8] anthropometry,[9] hormone factors[10] and likely alcohol consumption.[11]

Other factors may also influence melanoma development and contribute to its steady increase. Results from etiological studies indicate that exposure to and use of commonly prescribed drugs may represent such a factor (see online supplementary tables S1–S3). Drug safety has high priority and the European Medicines Agency has recently improved their systems, Exploring and Understanding Adverse Drug Reactions (EU-ADR) in the European Union, for active surveillance of adverse drug events. However, the EU-ADR is not ideal for capturing adverse events with long latency, such as cancer, because long-term monitoring is not part of the drug programme. Similar limitations apply for the US Food and Drug Administration (FDA). Consequently, knowledge on the possible carcinogenicity of marketed drugs is sporadic or lacking.

Pharmacoepidemiological studies and meta-analyses have contributed to establishing evidence of the carcinogenicity of drugs. Since 1970, IARC has performed comprehensive and systematic reviews of animal, laboratory, mechanistic and epidemiological studies to evaluate the carcinogenicity of drugs. Group 1 agents are those considered carcinogenic to humans, whereas groups 2a and 2b are agents with probable and possible carcinogenic effects, respectively.[12] However, many commonly used drugs have not been evaluated due to lack of long-term monitoring.

Some drugs can have skin carcinogenic potential, directly through a biological mechanism of the drug itself, which may include functional alterations of the immune system and the tumour microenvironment, and/or through an interaction with UV exposure, resulting in increased photosensitivity.[13 14] Drugs that could play a role in melanoma development through such mechanisms include some cardiovascular, antidepressants and immunosuppressive drugs although present studies do not show unanimous results (see online supplementary tables S1–S3). From 2005 to 2015, the number of people in Norway prescribed cardiovascular drugs rose from over 800 000 to over 1 000 000 (excluding inpatient use). The same numbers were 275 000 to about 330 000 for antidepressants and 26 000 to 55 000 for immunosuppressive drugs.[15 16]

The results of most studies warrant the need for further analyses with more detailed information on drug use and confounders to elucidate relations between these drug types and cancer.[17] Whether or not any drugs of these types have an association with the incidence of melanoma is highly important as the number of people receiving these drugs is increasing.

Cardiovascular drugs

Several types of cardiovascular drugs, including β-blocking agents, diuretics, ACE inhibitors (ACEi) and angiotensin receptor blockers (ARBs), may influence melanoma development (see online supplementary table S1). A biological basis for the role of β-blockers in melanoma progression exists, as melanoma tissue expresses both β1- and β2-adrenoreceptors. These, in turn, are known to stimulate the production of vascular endothelial growth factor, interleukin-6 and interleukin-8, which promote angiogenesis and tumour growth.[18] Long-term exposure to β-blockers has been associated with a reduced risk of melanoma progression,[19] melanoma recurrence and death.[20 21] On the other hand, a meta-analysis of studies found that β-blockers and diuretics might be positively associated with melanoma,[22] which has been supported by a recent meta-analysis of cohort studies, case–control studies, and randomised clinical trials.[17]

Diuretics have been shown to have photosensitising potential[23] and use of the diuretics indapamide and thiazide has been found to increase the risk of melanoma[22 24–26] though no such association was found in a recent meta-analysis.[17] Another recent analysis regarding the use of the diuretic hydrochlorothiazide found no association with melanoma in general, stratification by histological subtype however, revealed positive associations with the subtypes nodular and lentigo melanoma.[27] Use of statins however, another prominent drug group, has been associated with decreased melanoma progression.[28]

ACE may also be involved in cancer processes through regulation of cell proliferation and migration.[29] It remains unclear, whether ACEi or ARBs influence melanoma development. A review of observational and interventional studies indicated that ACEi and ARBs positively affect survival in melanoma patients.[30] A recent meta-analysis, however, found that neither ACEi nor ARBs were associated with any form of skin cancer.[17]

Antidepressant drugs

In a comprehensive European case–control study of known and potentially new risk factors for skin cancer, stress, traumatic events and depression were identified as significant risk factors for melanoma.[31] This relation can result from the biological effects of stress but also raises the question of whether it is the result of other factors like associated drug use.

Laboratory and animal studies have found cancer-promoting effects of antidepressants[32] while for melanoma, in particular, few studies exists (see online supplementary table S2). Major types of antidepressants include selective serotonin reuptake inhibitors (SSRI), non-selective monoamine reuptake inhibitors (NSMRI), monoamine oxidase inhibitors and tricyclic antidepressants (TCA). The SSRI sertraline displays cytotoxicity against human melanoma cell lines through downregulating the pro-survival molecule Akt that normally prevents cell death through apoptosis.[33] High-dose sertraline (75-fold to 100-fold higher than clinical doses) also has the capacity

to reduce protein synthesis and thus cell proliferation, giving it antineoplastic properties.[34]

Fluoxetine, another SSRI, has been found to induce melanogenesis in melanoma cell lines in vitro and in vivo,[35] and it is associated with an increased number of brain metastases from breast cancer in mice.[36] On the other hand, animal studies have demonstrated that fluoxetine significantly inhibits melanoma tumour growth and melanoma-induced oxidative changes through antioxidant activity.[37 38] The TCAs amitriptyline, nortriptyline and clomipramine have previously displayed an ability to inhibit the growth of melanoma cell lines and primary cell cultures in vitro.[39] The TCA desipramine is also demonstrated to inhibit melanoma tumour growth in vivo.[40]

Immunosuppressive drugs

Immunosuppressive drugs are used to prevent rejection following organ transplantation and for treatment of autoimmune disorders. These drugs have several well-documented side effects, of which infections and cancer are the most frequent due to the nonspecific nature of the immune suppression.[41] A well-known side effect is significantly increased risk of non-melanoma skin cancer,[42] but a positive association with melanoma risk and mortality have also been observed (see online supplementary table S3).[43]

A systematic review of the FDA adverse events reporting system and of medical records detected a significant association between tumour necrosis factor-α inhibitors and increased melanoma risk. The drugs identified as having an association with melanoma were the monoclonal antibodies, such as infliximab, adalimumab and golimumab, as well as the receptor fusion protein etanercept.[44] Glucocorticoids, another group of immunosuppressive agents, have been found to inhibit melanoma growth.[45 46]

The antiproliferative agent azathioprine causes accumulation of 6-thioguanine in DNA. These components are thought to work synergistically with UVA radiation to generate reactive oxygen species with mutagenic potential.[47] This propensity to increase UV-induced DNA damage is suggested to be responsible for the development of melanoma in users of azathioprine.[48]

A large and comprehensive population-based study using nation-wide registry data provides a unique opportunity to explore the impact of the drug types in question on melanoma risk. To our knowledge, a similar study has not been conducted, making the current research question a significant matter for public health systems worldwide.

Aims and hypothesis

The central hypothesis of this project is that use of cardiovascular, antidepressant and immunosuppressive drugs increases the risk of melanoma. With this study protocol, we propose a population-based case–control study with the aim of examining this hypothesis with the following questions:

1. Is use of prescribed cardiovascular drugs (in particular diuretics) associated with melanoma risk?
2. Is use of prescribed antidepressants associated with melanoma risk?
3. Is use of prescribed immunosuppressive drugs and/or monoclonal antibodies associated with melanoma risk?

Methods and analysis

This project will be carried out by merging data from four Norwegian national population-based registries (figure 1) with complete and high-quality data due to mandatory reporting by law. The unique personal identification number (PIN) issued to all Norwegian residents on birth or immigration enables data linkage across the registries. The study sample will encompass approximately 14 000 melanoma cases with 10 matched controls per case, alongside data regarding pre-diagnostically dispensed cardiovascular antidepressant and immunosuppressive
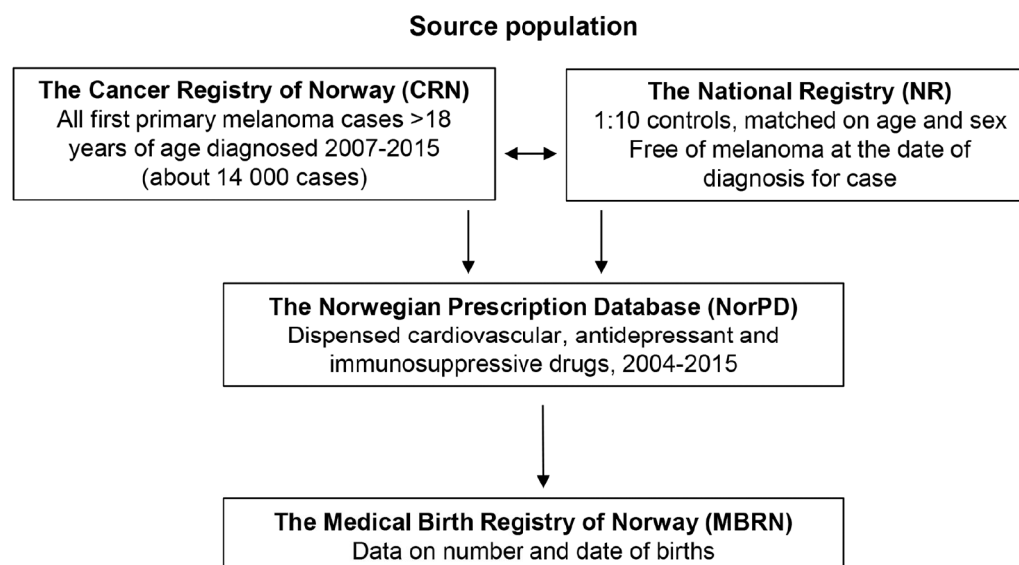
Source population

Figure 1 A diagram illustrating the source population and the data to be obtained from each of the four nation-wide registries.

drugs, including data regarding number and dates of child births.

Patient and public involvement

As the study proposed by the protocol in question is register based, the research question and outcome measures were not informed by any specific patient priorities, experiences or preferences. Rather, their formulation was based on our own priorities for patient benefit and result interpretation. The case–control study described by the protocol uses only data from nation-wide population-based registers and thus will not include a recruitment process for patients, who will not be involved in neither the design nor conduct of the study. All results will be distributed via the news media, relevant patient and drug user groups, as well as peer-reviewed journals and scientific conferences. The study described by the protocol in question is not a randomised control trial and will not have measures of intervention that could burden patients in any way assessable.

The registries

The Cancer Registry of Norway (CRN) has registered information on all cancers diagnosed in Norway since 1953. The registry receives data from several independent sources (medical practitioners, pathology laboratories and the Cause-of-Death Registry) ensuring complete and up-to-date high-quality data.[49] Cancer diagnoses are recorded using the International Classification of Disease version 10. For our analyses, we will obtain the following data on all first-time melanoma cases, diagnosed in the age group 18–85 years between 2007 and 2015: sex, age at diagnosis, date of diagnosis, tumour location, histopathological factors (histological type, anatomic location (see online supplementary table S4), Breslow thickness (since 2008), clinical stage and ulceration) and place of residence. Case-by-case data regarding Breslow thickness is missing from all diagnoses in 2007 but will be included through imputation in order to study Breslow thickness as an outcome.

The National Registry contains information on births, citizenship, change of address and migration to and from Norway with dates, for all citizens, which allows for the sampling of general population controls and tracking of all study subjects. The Norwegian Prescription Database (NorPD) contains information on all prescribed medications (reimbursed or not), dispensed at pharmacies to individual patients treated in ambulatory care from 1 January 2004 in the entire Norwegian population (5.3 million individuals in 2018). In NorPD, the information available for each dispensed drug is the Anatomical Therapeutic Chemical (ATC) classification code, substance name, trade name, pharmaceutical formulation, strength, package size, number of packages, amount dispensed in Defined Daily Doses, reimbursement code and dispensing date.[50]

Drugs supplied in hospitals and nursing homes are not included at the individual level in NorPD. All drugs dispensed are classified according to the WHO ATC classification.[51] For the purpose of our analyses, we will obtain information on use of cardiovascular (and in particular diuretic) drugs (ATC code: C01-C10), antidepressant drugs (ATC code: N06A), immunosuppressive (ATC code: L04) drugs (see online supplementary table S4), as well as the use of other drug types. All drugs in question are prohibited for sale in Norway without an associated prescription from a physician. The drugs of each type considered for the analysis will be limited to those where the amount of available patient user data can facilitate statistically significant data analysis. Data from region-specific UV measurement stations will be obtained from the Norwegian Radiation Protection Authority to calculate ambient lifetime cumulative UV dose according to county of residence at the time of diagnosis.[52] The Medical Birth Registry of Norway (MBRN) was established in 1967 and has since recorded information on all deliveries in Norway. Data to be obtained for all cases and controls are number and dates for births experienced until the point of diagnosis (cases) or index date (controls).

Study design

Using a nested case–control design, we will explore the melanoma incidence and level of multiple drug exposures in melanoma cases and controls. Furthermore, we will investigate whether drug use is related to melanoma risk, as well as to histological subtype, clinical stage, Breslow thickness, ulceration and ambient UV exposure of residence through stratified analyses. Cases will consist of all first primary histologically verified melanomas (18–85 years) diagnosed in Norway in the period 2007–2015 (figure 2). In all, 10 controls per case (1:10) will randomly be selected from the general population, alive and free of cancer at the date of diagnosis (index
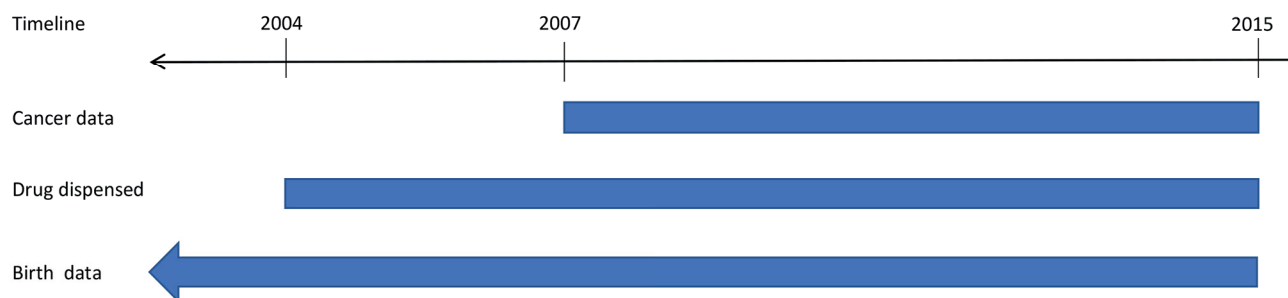
Figure 2 A timeline illustrating from which time periods the relevant data are to be obtained for the study.

Table 1 Overview of case, control and matching criteria for the study sample

Case criteria	Study criteria
Cases	~14 000
Verification	Histological or cytological verified melanoma (ICD-10: C43)
Definition	Norwegian inhabitants with a diagnosis of invasive melanoma without a history of cancer
Age at diagnosis	18–85 years
Year of diagnosis	2007–2015
Sex	Male and female
Control criteria	
Controls	~140 000 (1:10 matching)
Definition	Alive, resident in Norway with no history of cancer before respective case diagnosis
Selection	Random sampling within matching criteria (with replacement) from a pool of available population
Matching criteria	
Sex	Same sex as case
Age at diagnosis	Same year of birth as case
Index date	Alive and free of cancer at the date of diagnosis (case)

ICD-10, International Classification of Disease version 10.

date) for the case, and matched on sex and year of birth (risk set sampling). Table 1 gives the description of case, control and matching criteria.

Any case which is found to have two or more simultaneous diagnoses of melanoma will be removed from the main analysis in addition to their respective controls. This subgroup may, however, constitute an additional subject of investigation given that its numbers can facilitate a statistical analysis of sufficient power. Exposure to a particular drug or drug group among all cases and controls will be assessed from drugs dispensed as recorded in NorPD from 2004 to 2015 (figure 2). First, drug exposure will be defined as chronic drug use, that is, the dispensing of a drug which covers at least 2 years of use before the index date. Second, the cumulative dose will be assessed based on the number of prescriptions, total dose and duration of use, for each drug group. Third, drug exposure will be modelled as a time-dependent exposure by categorising the drug use at each time point as nonuser, user and past user. NorPD has registered dispensed prescription drugs from 1 January 2004. To account for the uncertainty of drug use before this date, we will apply a 6-month quarantine from 1 January 2004 to 30 June 2004. Thus, we will exclude all individuals with drug use within this time frame. Alternatively, we will use all registered dispensed drugs after 1 January 2004 and adjust for drug use within the time period from 1 January 2004 to 30 June 2004. Drug

groups will be categorised into therapeutic subgroups (ATC second level). These subgroups will additionally be categorised by pharmacological subgroups (ATC fourth level) and chemical substances (ATC fifth level) to account for the potential confounding introduced by the different indications for which the drugs of interest can be given.[53] Thus, where applicable with regard to statistical power, this will allow for the comparison of effects between subgroups and enable the use of active comparators as controls for specific agents of interest. To reduce confounding by indication, an additional covariate pertaining to the dispensation of other drug types prior to index date in addition to cardiovascular, antidepressant and immunosuppressive drugs will be implemented as a proxy for general healthcare usage among cases and controls.

Accounting for a certain latency period is prudent when assigning cancer development to some drug types as it reduces the possibility of reverse causation bias. On the other hand, certain drugs may have cancer-promoting properties which mediate late steps in the carcinogenesis.[54] Other studies have also demonstrated the potential for relatively immediate effects of interventions designed to mediate the risk of melanoma.[55] To account for this, the analyses will be conducted with and without consideration for a 1-, 3- and 5-year latency period between drug use and melanoma diagnosis. Additionally, as a lag period after drug discontinuation covers the latent period in which the effects of the drug in focus may still manifest, the time after drug discontinuation will also be considered time at risk with regard to attributing carcinogenic or anticarcinogenic properties to drugs.

Statistical methods
As the study will have a nested case–control design with risk set sampling (1:10 matching), conditional logistic regression analysis will be the main statistical method, estimating ORs and 95% CIs for the association between melanoma and the drug in focus. Drug use will be modelled as a binary (chronic drug use) and continuous (cumulative dose) variable (see above).

In the analyses of drug use in relation to anatomic location of the tumour, we will test whether exposure–disease associations differ by sites by a contrast test. The same approach will be used in a stratified analysis of drug use and its associations with histopathological subtypes, clinical stage, Breslow thickness and ulceration (since 2008; in T categories[56]). We will also perform a linear regression analysis, using the Breslow thickness of melanoma as a continuous outcome variable among cases only. Due to the skewed distribution of Breslow thickness, $\log_e$-transformation will be used and back-transformed estimates (geometric means) will be presented.[9]

We will adjust for residential ambient UV exposure according to lifetime cumulative UV dose.[9] We will also categorise region of residence as urban or rural areas to indicate dermatologist availability. Number of births is also a potential covariate in the analyses. We will test for

Table 2 The minimum OR detectable according to proportion of controls exposed to a particular drug type, using a power of 80% and a significance level of 0.05

Proportion of exposed controls (%)	OR	Number of cases	Number of controls	Total study population
5	1.1	18 902	189 020	207 922
5	1.2	4904	49 040	53 944
5	1.3	2257	22 570	24 827
10	1.1	10 041	100 410	110 451
10	1.2	2622	26 220	28 842
10	1.3	1214	12 140	13 354
20	1.1	5722	57 220	62 942
20	1.2	1513	15 130	16 643
20	1.3	709	7090	7799

relevant interactions such as sex/drugs, urban or rural residence/drugs as well as number of births/drugs. The significance level will be set to 5% and all statistical analyses will be performed using the R Statistical Software Package (V.3.5.1).[57]

Power and sample size calculations

The statistical power was set to 80% with a significance level of 5%. Calculations were performed using R. Table 2 shows the minimum OR detectable for different sample sizes under the assumption that various proportions of controls are using a particular type of drug. Due to the size of the study samples for each study (n=154 000) including 14 000 melanoma cases, we have enough statistical power to detect an OR of at least 1.2, assuming that 5% of the controls are exposed to the drug in question. Alternatively, an OR of 1.1 can also be achieved if at least 10% or 20% of controls have been exposed to the particular drug in question.

Analysis plan

In order to test the hypotheses above, the following analyses will be conducted:

1.1: A matched case–control analysis of overall melanoma risk according to the exposure and level of use of prescribed cardiovascular drugs (diuretics in particular).

1.2: A matched case–control analysis of melanoma risk stratified by anatomic site, histopathological subtype, clinical stage, Breslow thickness, ulceration and residential ambient UV exposure, according to the exposure and level of use of prescribed cardiovascular drugs (diuretics in particular).

2.1: A matched case–control analysis of melanoma risk according to the exposure and level of use of prescribed antidepressant drugs.

2.2: A matched case–control analysis of melanoma risk stratified by anatomic site, histopathological subtype, clinical stage, Breslow thickness, ulceration and residential ambient UV exposure, according to the exposure and level of use of prescribed antidepressant drugs.

3.1: A matched case–control analysis of melanoma risk according to the exposure and level of use of prescribed immunosuppressive drugs and/or monoclonal antibodies.

3.2: A matched case–control analysis of melanoma risk stratified by anatomic site, histopathological subtype, clinical stage, Breslow thickness, ulceration and residential ambient UV exposure, according to the exposure and level of use of prescribed immunosuppressive drugs and/or monoclonal antibodies.

4: A linear regression analysis examining the Breslow thickness of melanoma as a continuous outcome, among cases only, according to the exposure and level of use of prescribed drugs.

Project strengths and limitations

Each analysis relies on high-quality data collected from nation-wide population-based health registries from 2004 to 2015, with mandatory reporting and linkage secured by the PINs. This level of detail lends itself well to this prospective case–control study and allows us to take into account a wide range of variables for a high level of resolution in the statistical analyses. While recall bias represents a frequent limitation to the case–control design, all exposure data for the analysis will have been collected before the outcome. Hence, the use of prospectively collected high-quality data, without the need for personal recollection, eliminates the risk of recall bias.

While we will assume that drugs were used on the same date at which they were dispensed from the NorPD, it is not known, for certain, whether the drugs in question were used at this time. However, because only information pertaining to drug dispensation and purchase by patients is recorded in the NorPD, primary nonadherence is not an issue.[58] The NorPD only records information on all prescribed drugs dispensed to individual patients from all pharmacies in Norway, excluding nonprescribed drugs and drugs dispensed to inpatients in hospitals or institutions. However, given the size and quality of our data from the general population, it is unlikely that this limitation will significantly influence the main results of our study. Additionally, as reporting to the respective registers is mandatory by law, the problem of selection bias is therefore negligible. Underlying indications for drug use might influence the risk of melanoma and may introduce potential confounding by indication. In addition to the use of cardiovascular, antidepressant and immunosuppressant drugs, we will account for the use of other drug types in our analyses, which will simultaneously act as a proxy indicator of potential differences in healthcare usage.

The main limitation is the potentially short latency time between drug use and melanoma diagnosis that this study allows for. The NorPD holds individual data on prescribed drugs dispensed to individuals since 1 January 2004, which can result in a short latency time for cancer development and detection throughout 2007–2015. The exposure window for most cancer–drug associations is unknown, though a quantitative analysis of the

genetic evolution of pancreatic cancer found a 17-year gap between the initial carcinogenic mutation and the acquisition of metastatic capabilities by the primary tumour.[59] The time between initial carcinogenesis and clinical detection of many cancers is also assumed to be long (10–30 years in some cases), and cancer is thus not an immediate effect of drug exposure.[13] The long period of cancer development, the latency of any carcinogenic and antineoplastic drug effects and unknown biological mechanisms of efficacy all contribute to the considerable time it takes to fully elucidate potential drug–cancer relationships. Additionally, while we will adjust for residential ambient UV exposure, we will not be able to account for other UV exposure variables such as recreational sun exposure, sunburns (as a marker of episodes of severe acute UV exposure) or indoor tanning. Neither will we be able to take phenotypic characteristics (fair complexion, freckles and nevi), socioeconomic variables (eg, education, occupation), healthcare utilisation, comorbidity, postmenopausal hormone use and anthropometric factors into account, which may represent confounding sources of individual-level exposure.

ETHICS AND DISSEMINATION

The linkage key for the 11-digit PINs will be stored and governed by a third party unavailable to the research team. All data management and analyses will be conducted on encrypted data with no individual persons identified.

This project can generate new and important knowledge on risk factors for melanoma and about melanoma aetiology, for better and more targeted prevention measures both in Norway and internationally. Our results can be of high importance for users of prescribed drugs and for the design of public health campaigns and future surveillance programmes, specifically addressing patients with a risk profile that predisposes for development of melanoma.

All results will be published in international peer-reviewed journals and presented at national and international conferences. The results will also be communicated directly to relevant user groups such as the Norwegian Cancer Society, The Norwegian Melanoma Association and other interest groups for patients that would be dependent on the drugs in question. Annual Norwegian conferences and seminars will serve as additional platforms for the distribution of knowledge to clinicians and researchers. Furthermore, a project-specific website, social media and other potential channels will also serve as platforms to distribute relevant results to patients and the general population.

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Paper I

ORIGINAL RESEARCH

Use of Antidepressants and Risk of Cutaneous Melanoma: A Prospective Registry-Based Case-Control Study

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Purpose: Melanoma is the cancer with the most rapidly rising incidence rate in Norway. Although exposure to ultraviolet radiation (UVR) is the major environmental risk factor, other factors may also contribute. Antidepressants have cancer inhibiting and promoting side effects, and their prescription rates have increased in parallel with melanoma incidence. Thus, we aimed to prospectively examine the association between use of antidepressants and melanoma by using nation-wide data from the Cancer Registry of Norway, the National Registry, the Norwegian Prescription Database and the Medical Birth Registry of Norway.

Patient and Methods: All cases aged 18–85 with a primary cutaneous invasive melanoma diagnosed during 2007–2015 (n=12,099) were matched to population controls 1:10 (n=118,467) by sex and year of birth using risk-set sampling. We obtained information on prescribed antidepressants and other potentially confounding drug use (2004–2015). Conditional logistic regression was used to estimate adjusted rate ratios (RRs) and 95% confidence intervals (CIs) for the association between overall and class-specific use of antidepressants and incident melanoma.

Results: Compared with ≤1 prescription, ≥8 prescriptions of antidepressants overall were negatively associated with melanoma (RR 0.81 CI 0.75–0.87). Class-specific analyses showed decreased RRs for selective serotonin reuptake inhibitors (RR 0.82 CI 0.73–0.93) and mixed antidepressants (RR 0.77 CI 0.69–0.86). The negative association was found for both sexes, age ≥50 years, residential regions with medium and highest ambient UVR exposure, all histological subtypes, trunk, upper and lower limb sites and local disease.

Conclusion: Use of antidepressants was associated with decreased risk of melanoma. There are at least two possible explanations for our results; cancer-inhibiting actions induced by the drug and less UVR exposure among the most frequent users of antidepressants.

Keywords: antidepressants, melanoma, prescription drugs, pharmacoepidemiology, registry-based

Summary

Melanoma incidence rates are high and rising in many fair-skinned populations, and are mostly caused by excessive sun exposure. However, use of prescribed drugs may influence the risk as some drugs can affect both skin sensitivity to sun exposure and immune responses. Antidepressants are shown to have effects that can inhibit or promote cancer development and the prescription rates have increased in parallel with melanoma rates. We therefore aimed to study the use of antidepressants and melanoma risk.

Compared to non-users, the most frequent users had decreased melanoma risk that was found for both sexes, though the decrease was greater in men, for ages ≥50 years, in regions with the highest ambient ultraviolet radiation (UVR) exposure, for all histological

subtypes, for all body sites except the head and neck and for local disease. There are at least two possible explanations for our results; anti-cancer actions of antidepressants and less sun exposure among the most frequent users of antidepressants.

Introduction

The incidence of cutaneous melanoma (hereafter melanoma) is increasing in Caucasian populations worldwide. Since the year 2000, melanoma has become the most rapidly growing malignancy in Norway,[1] which ranks amongst the top five countries worldwide in age-standardized melanoma incidence and mortality rates.[2] Approximately three-quarters of all melanoma cases are estimated to be attributable to ultraviolet radiation (UVR) exposure.[3] However, the development of melanoma is a multifactorial process which also depends on individual susceptibility, including skin sensitivity to UVR and number of nevi.[4] Other factors such as previous melanoma diagnosis,[5] family history of melanoma,[6] body anthropometry,[7] hormonal factors[8] and alcohol consumption[9] have also been implicated. In addition, a comprehensive European case-control study found that stress, traumatic events and depression were significantly associated with increased melanoma risk.[10] Possible causal associations could result from the biological effects of stress, which promotes cancer development,[11,12] or from associated use of antidepressants. Over the last decades, prescription of antidepressants has increased in several countries,[13,14] including in Norway.[15,16] Results from preclinical studies show both cancer-promoting and inhibiting side effects of antidepressant agents,[17–19] raising the question of whether the use of such drugs influences cancer risk.

Observational studies in humans have examined the associations between antidepressant use and several cancers. Early studies indicated a positive association for some cancers, while the predominant finding in more recent studies indicates a negative association.[18] For melanoma, preclinical studies indicate inhibiting side-effects of antidepressant agents.[20–22] Though observational population-based studies concerning this relationship appear to be lacking.[23,24] Thus, in this nationwide case-control study we aimed to prospectively examine the association between use of antidepressants and melanoma development, to assess whether such use might influence the melanoma rate.

Materials and Methods

The study has a nested case-control design and uses nationwide population-based registry data that includes the entire Norwegian population, aged 18–85, in the time period 2004–2015 (3.9 million people). The data were obtained from the Cancer Registry of Norway (CRN), the Norwegian Prescription Database (NorPD), the National Registry and the Medical Birth Registry of Norway. Data were linked using the unique personal identification numbers (PIN) assigned to all Norwegian residents. The study design and data collection have been described in detail previously.[25]

Selecting Cases and Controls

The CRN has recorded cancer diagnoses compulsorily by law since 1953, and the completeness exceeds 98%.[1] We obtained information about all melanoma cases in the age group 18–85 years that were diagnosed with a first primary melanoma during 2007–2015 (n=12,099). In the CRN, cancer diagnosis is registered according to the International Classification of Diseases (ICD) of oncology 3rd edition (ICD-O-3) and reported according to the ICD 10th Revision (C43). Tumor site registered according to ICD-7 was categorized as head/neck (190.0), trunk (190.1, 190.7), upper limb (190.2), lower limb (190.3, 190.4), other (190.5, 190.6, 190.8) and unspecified (190.9). Histological subtype (registered according to the ICD-O-3) was categorized as superficial spreading melanoma (SSM; 87433), nodular melanoma (NM; 87213) and other subtypes (87423, 87443, 87453/87803/87613, including unspecified 87203). Lastly, based on information about metastases, CRN records stage at diagnosis as local disease (no metastases), regional metastasis (metastases in regional lymph nodes, satellites and in transit metastases), distant metastasis (organ metastases and non-regional lymph node metastases) and unspecified. Utilizing risk set sampling, 10 controls for each case were matched on sex and year of birth (n=118,467). Controls had to be alive, residents in Norway and free of cancer at the date of diagnosis for their respective cases (index date), though could develop cancer thereafter.

Assessment of Antidepressant Use

The NorPD records all prescribed medications dispensed at Norwegian pharmacies to non-institutionalized individuals since 01.01.2004.[26] Drugs dispensed are classified according to the international Anatomical Therapeutic Chemical (ATC) classification system.[27] A record of each dispensation is made with information about the patient, prescriber and medication.[26] Information in our study from 2004–2015 includes the ATC classification code and defined daily doses (DDD) dispensed per prescription in accordance with the World Health Organization Collaborating Centre for Drug Statistics Methodology. Antidepressants were defined as any drug included in the ATC group N06A and

classified as selective serotonin reuptake inhibitors (SSRI; N06AB), tricyclic antidepressants (TCAs; N06AA) and other antidepressants (N06AF, N06AG and N06AX). Use of antidepressants from more than one class was defined as mixed use.

Use of antidepressants was categorized according to number of prescriptions: ≤1 prescription, 2–7 prescriptions and ≥8 prescriptions. The prescriptions may have been obtained at separate or the same dates. Cumulative dose was quantified for each individual based on the total number of DDDs and categorized as non-users and in quartiles (1–91 DDD; 92–365 DDD; 366–1460 DDD; ≥1461 DDD) for the whole sample, for antidepressants overall and for each class of antidepressant. Duration of use was defined as the time between the first and last prescription for each individual, and categorized as ≤5 years and >5 years.

To reduce the possible impact of reverse causation, prescriptions of antidepressants dispensed within a year prior to the date of diagnosis (cases)/index date (controls) were disregarded, as well as prescriptions dispensed after the diagnosis/index date.

Potential Confounders

Certain drugs that affect skin sensitivity to UVR or immune mechanisms could influence the risk of melanoma.[28–31] To take use of other drugs into account, information about ever use of immunosuppressive drugs (yes/no), ever use of cardiovascular drugs (yes/no) and >1 prescription dispensed of other drugs (yes/no) were obtained from NorPD.

The UVR doses increase from northern to southern Norway. Information about the region of residence was obtained from the CRN (cases) and NorPD (controls) and categorized according to cumulative doses of ambient UVR exposure.[32] First in a five-level variable: northern Norway, central Norway, southwestern Norway, southeast inland, and southeast coast.[7] In addition, we categorized it in a three-level variable as low (northern Norway), medium (western and central Norway) and highest (eastern and southern Norway).[33] Cases and controls without information about the place of residence were excluded (67 cases and 3257 controls).

Number of children has been inversely associated with melanoma risk, suggesting a potential role of female sex hormones,[34,35] or of factors related to parity like socioeconomic status and sunbathing habit.[34] Thus, information about number of children (prior to diagnosis/index date) for all women was obtained from the Medical Birth Registry and categorized as 0, 1–3 and >3 children.

Permission to conduct the study was provided by the Norwegian Data Inspectorate, the Regional Committees for Medical and Health Research Ethics and each of the registries that contributed data.

Statistical Analyses

Conditional logistic regression was used to assess the relation between antidepressant use and melanoma risk. Rate ratios (RRs) with 95% confidence intervals (CIs) were estimated, as the collection of controls was done by risk-set sampling.[36] Stratified analyses were also performed by classes of antidepressant prescribed (SSRI, TCAs, other and mixed). All analyses were adjusted for sex and birth year by design, for use of other drugs and residential ambient UVR region. For women, analyses were conducted with and without adjustment for number of children, but the multivariable results are presented without this additional adjustment, since the difference in results was negligible.

To test for trends in prescription number and cumulative dose, the median of each category was used. We tested for interactions between number of prescriptions and the relevant variables: sex, age at diagnosis/index date, ambient UVR region (in three categories) and number of children (for women only) with a likelihood ratio test. Stratified analyses were directed by prior biological and statistical knowledge. RRs were also estimated for the association between number of antidepressant prescriptions and melanoma, stratified by sex, age at diagnosis/index date and residential ambient UVR exposure. Furthermore, we estimated RRs for the associations between number of antidepressant prescriptions and melanoma site, histological subtype, and clinical stage at diagnosis.

In supplementary analyses, RRs were estimated for the association between melanoma and duration of use with a short (≤5 years) and long (>5 years) timeframe, and for the associations between melanoma subtype and number of prescriptions by antidepressant classes.

All statistical analyses were conducted using the statistical software package R (version 3.5.1).[37] The significance level was set to 5%, and all tests were two-sided.

Results

The final study sample consisted of 130,566 individuals. This included 5985 male and 6114 female melanoma cases diagnosed during 2007–2015 with 58,269 and 60,198 population controls, respectively. The majority of cases and controls were ≥50 years of age and resided in the southern/eastern region of Norway (Table 1). Most tumors were located at the

Table 1 Characteristics of Cases and Controls in the Study Cohort

Characteristics	Cases (n=12,099) No. (%)	Controls (n=118,467) No. (%)
Sex		
Men	5985 (49.5)	58,269 (49.2)
Women	6114 (50.5)	60,198 (50.8)
Age at Diagnosis/Index Date, Years		
<50	3160 (26.1)	30,319 (25.6)
50–69	5615 (46.4)	55,236 (46.6)
≥70	3324 (27.5)	32,912 (27.8)
Residential Ambient UVR[a] Exposure		
Low (northern Norway)	716 (5.9)	11,895 (10.0)
Medium (central/western Norway)	1724 (14.3)	19,429 (16.4)
Highest (southern/eastern Norway)	9659 (79.8)	87,143 (73.6)
Parity (Women Only)		
No children	1861 (30.4)	18,984 (31.5)
1–3 children	3894 (63.7)	36,901 (61.3)
>3 children	359 (5.9)	4313 (7.2)
Tumor Site		
Head/neck	1314 (10.9)	-
Trunk	5657 (46.7)	-
Upper limb	1559 (12.9)	-
Lower limb	2867 (23.7)	-
Other sites	52 (0.4)	-
Unspecified site	650 (5.4)	-
Histological Type		
Superficial spreading melanoma	6654 (55.0)	-
Nodular melanoma	2076 (17.2)	-
Other	3369 (27.8)	-
Clinical Stage		
Local disease	9828 (81.2)	-
Regional metastasis	572 (4.7)	-
Distant metastasis	635 (5.3)	-
Unspecified	1064 (8.8)	-
Use of Antidepressants		
I. Number of Prescriptions		
≤1	10,597 (87.6)	102,154 (86.2)
2–7	663 (5.5)	6612 (5.6)
≥8	839 (6.9)	9701 (8.2)
II. By Antidepressant Classes		
Non-user[b]	10,185 (84.2)	97,823 (82.6)
SSRI[c]	699 (5.8)	7358 (6.2)
TCA[d]	375 (3.1)	3749 (3.2)
Other[e]	317 (2.6)	3486 (2.9)
Mixed[f]	523 (4.3)	6051 (5.1)
III. By Cumulative Dose, DDD[g]		
0	10,185 (84.2)	97,823 (82.6)

(Continued)

Table 1 (Continued).

Characteristics	Cases (n=12,099) No. (%)	Controls (n=118,467) No. (%)
1–91	524 (4.3)	5630 (4.8)
92–365	454 (3.8)	4764 (4.0)
366–1460	508 (4.2)	5239 (4.4)
≥1461	428 (3.5)	5011 (4.2)
Ever Use of Other Drugs		
Immunosuppressant drugs[h]	1862 (15.4)	18,511 (15.6)
Cardiovascular drugs[h]	5951 (49.2)	57,264 (48.3)
Other drugs[i]	5379 (44.5)	53,033 (44.8)

Notes: [a]Ultraviolet radiation; [b]0 prescription; [c]Selective serotonin reuptake inhibitors (N06AB); [d]Tricyclic antidepressants (N06AA); [e]Other antidepressants (N06AF, N06AG and N06AX); [f]Use of more than one class of antidepressants; [g]Defined daily dose; [h]Includes mixed use with other drugs; [i]Includes use of other drugs than immunosuppressant or cardiovascular drugs.

trunk (47%) and lower limb (24%). SSM was the most common histological subtype (55%), and the majority were diagnosed in a local stage (81%).

The overall use of antidepressants was 12% among cases and 14% among controls. Women represented half the proportion of users overall (51% among cases and 51% among controls) but represented a higher proportion in those with ≥8 prescriptions (69% and 66%, respectively) and 2–7 prescriptions (63% and 61%, respectively). The most commonly prescribed antidepressant class was SSRIs (51%), where escitalopram (44%) was the most common drug.

When compared with ≤1 prescription, ≥8 prescriptions of any type of antidepressants were associated with a 19% reduced risk of melanoma with a negative trend (Table 2). Analysis stratified by duration of use showed significantly lower risk for 2–7 prescriptions (RR 0.52 95% CI 0.38–0.74) and ≥8 prescriptions (RR 0.79 95% CI 0.72–0.87) among long-term users (>5 years). Significantly lower risk was only found for ≥8 prescriptions (RR 0.82 95% CI 0.74–0.92) among short-term users (≤5 years) (Supplementary Table S1). Analyses stratified by antidepressant classes showed an 18% and 23% lower melanoma risk for persons with ≥8 prescriptions of SSRIs and mixed antidepressants, respectively, compared to ≤1 prescription (Table 2). For the class of other antidepressants, a significantly negative trend was seen, despite non-significant effect estimates, while for TCA, no associations were found.

Compared to the lowest quartile of cumulative dose (1–91 DDD), no significant association with melanoma risk was found for increasing quartiles (Table 3). However, a significant trend was found and non-use (0 DDD) was associated with a 15% increased risk. In addition, a reduced

Table 2 Rate Ratios (RRs) with 95% Confidence Intervals (CIs) for Melanoma Incidence by Number of Prescriptions of Antidepressants Overall and Stratified by Class of Antidepressants

Number of Prescriptions	No. Case/Controls	RR (95% CI)[a]	P$_{trend}$[f]
Overall			
≤1	10,597/102,154	1.00 (reference)	
2–7	663/6612	0.94 (0.87, 1.03)	
≥8	839/9701	0.81 (0.75, 0.87)	<0.001
By Antidepressant Classes			
SSRI[b]			
≤1	4736/45,455	1.00 (reference)	
≥8	289/3280	0.82 (0.73, 0.93)	0.003
TCA[c]			
≤1	2782/26,637	1.00 (reference)	
≥8	107/1100	0.90 (0.73, 1.10)	0.295
Other[d]			
≤1	2679/25,576	1.00 (reference)	
≥8	84/967	0.81 (0.65, 1.02)	0.036
Mixed[e]			
≤1	3838/36,528	1.00 (reference)	
≥8	359/4354	0.77 (0.69, 0.86)	<0.001

Notes: [a]Adjusted for sex and birth year (by design) and ever use of other drugs and residential ambient ultraviolet radiation; [b]Selective serotonin reuptake inhibitors (N06AB); [c]Tricyclic antidepressants (N06AA); [d]Other antidepressants (N06AF, N06AG and N06AX); [e] Use of more than one class of antidepressants; [f]Trend test uses the median number of prescriptions for all prescription categories (≤1; 2–7; ≥8).

Table 3 Rate Ratios (RRs) with 95% Confidence Intervals (CIs) for Melanoma Incidence by Quartiles (Q) of Cumulative Defined Daily Dose (DDD) of Antidepressants Overall and Stratified by Class of Antidepressants

Cumulative Dose, DDD	No. Case/Controls	RR (95% CI)[a]	P$_{trend}$[f]
Overall			
0	10,185/97,823	1.15 (1.04, 1.26)	
Q1 (DDD 1–91)	524/5630	1 (Reference)	
Q2 (DDD 92–365)	454/4764	1.03 (0.90, 1.17)	
Q3 (DDD 366–1460)	508/5239	1.04 (0.91, 1.18)	
Q4 (DDD ≥1461)	428/5011	0.91 (0.80, 1.04)	<0.001
By Antidepressant Classes			
SSRI[b]			
0	4617/44,077	1.24 (0.99, 1.55)	
Q1 (DDD 1–91)	88/1030	1 (Reference)	
Q2 (DDD 92–365)	210/2171	1.12 (0.86, 1.46)	
Q3 (DDD 366–1460)	237/2227	1.23 (0.95, 1.59)	
Q4 (DDD ≥1461)	164/1930	0.99 (0.75, 1.28)	0.007
TCA[c]			
0	2628/25,104	1.08 (0.94, 1.23)	
Q1 (DDD 1–91)	242/2432	1 (Reference)	
Q2 (DDD 92–365)	57/619	0.92 (0.68, 1.25)	
Q3 (DDD 366–1460)	60/550	1.09 (0.81, 1.47)	
Q4 (DDD ≥1461)	16/148	1.07 (0.62, 1.83)	0.906
Other[d]			
0	2540/24,156	1.11 (0.94, 1.31)	
Q1 (DDD 1–91)	164/1714	1 (Reference)	
Q2 (DDD 92–365)	60/781	0.79 (0.58, 1.07)	
Q3 (DDD 366–1460)	56/555	1.05 (0.76, 1.46)	
Q4 (DDD ≥1461)	37/436	0.87 (0.60, 1.27)	0.077
Mixed[e]			
0	3838/36,528	1.69 (1.16, 2.45)	
Q1 (DDD 1–91)	30/454	1 (Reference)	
Q2 (DDD 92–365)	127/1193	1.65 (1.09, 2.50)	
Q3 (DDD 366–1460)	155/1907	1.28 (0.85, 1.92)	
Q4 (DDD ≥1461)	211/2497	1.33 (0.89, 1.98)	0.103

Notes: [a]Adjusted for sex and birth year (by design) and ever use of other drugs and residential ambient ultraviolet radiation; [b]Selective serotonin reuptake inhibitors (N06AB); [c]Tricyclic antidepressants (N06AA); [d]Other antidepressants (N06AF, N06AG and N06AX); [e]Use of more than one class of antidepressants; [f]Trend test uses the median defined daily dosage for all quartiles (0; 1–91; 92-365; 366-1460; ≥1461).

risk was found per increment of 100 DDD overall (P$_{trend}$ 0.001) (not shown). When stratifying by classes of antidepressants, no significant associations were found, except for a 69% increased risk among non-users and a 65% increased risk for a cumulative dose of 92–365 DDD when compared to the lowest DDD quartile of mixed antidepressant use (Table 3).

Compared to ≤1 prescription, those with ≥8 prescriptions of antidepressants had a significantly decreased risk of melanoma for specified tumor sites except the head and neck, other and unspecified sites (Table 4). Moreover, ≥8 prescriptions were associated with a decreased risk of all melanoma subtypes, with significant negative trends (P$_{trend}$ <0.001 (SSM); 0.017 (NM); 0.013 (other)). Supplementary analyses, stratified by antidepressant classes, showed that ≥8 prescriptions of SSRIs were associated with a decreased risk of SSM (RR 0.81 95% CI 0.69–0.96). Mixed antidepressants were associated with a decreased risk of SSM (RR 0.75 95% CI 0.65–0.87) and NM (RR 0.70 95% CI 0.52–0.94). (Supplementary Table S2). Lastly, ≥8 prescriptions of

antidepressants were associated with a 21% decreased risk for local stage melanoma, while no associations were found for other stages (Table 4).

Compared to ≤1 prescription, men and women with ≥8 prescriptions of any antidepressant had 27% and 14% reduced risk of melanoma, respectively (P$_{interaction}$ 0.029) (Table 5). No association was found for the youngest age group (<50 years), while a 24% and 21% reduced melanoma risk was found in the age groups 50–69 and ≥70 years,

Table 4 Rate Ratios (RRs) with 95% Confidence Intervals (CIs) of Melanoma Incidence for ≥8 Prescriptions of Antidepressants versus ≤1 Prescription, Stratified by Tumor Site, Histological Type, and Clinical Stage at Diagnosis

	No. Case/ Controls	≥8 Prescriptions vs ≤1 Prescription	
		RR (95% CI)[a]	P$_{trend}$[e]
Tumor Site			
Head/neck	1314/12,818	0.93 (0.75, 1.15)	0.468
Trunk	5657/55,032	0.79 (0.70, 0.88)	<0.001
Upper limb	1559/15,181	0.80 (0.66, 0.97)	0.022
Lower limb	2867/27,962	0.76 (0.65, 0.88)	<0.001
Other	52/511	0.99 (0.37, 2.64)	0.997
Unspecified	650/6354	1.07 (0.79, 1.45)	0.780
Histological Type			
SSM[b]	6654/64,763	0.80 (0.73, 0.89)	<0.001
NM[c]	2076/20,282	0.80 (0.67, 0.96)	0.017
Other[d]	3369/32,813	0.83 (0.72, 0.96)	0.013
Clinical Stage			
Local disease	9828/95,725	0.79 (0.73, 0.86)	<0.001
Regional metastasis	572/5569	0.89 (0.64, 1.24)	0.438
Distant metastasis	635/6209	0.91 (0.65, 1.27)	0.559
Unspecified	1064/10,328	0.98 (0.75, 1.28)	0.982

Notes: [a]Adjusted for sex and age (by design), ever use of other drugs, and residential ambient ultraviolet radiation; [b]Superficial spreading melanoma; [c]Nodular melanoma; [d]Includes all other histological types; [e]Trend test uses the median number of prescriptions in all three prescription categories (≤1; 2−7; ≥8).

Table 5 Rate Ratios (RRs) with 95% Confidence Intervals (CIs) of Melanoma Incidence for ≥8 Prescriptions of Antidepressants versus ≤1 Prescription, Stratified by Sex, Age at Diagnosis/Index Date and Residential Ambient Ultraviolet Radiation (UVR) Exposure

Variables	≥8 Prescriptions vs ≤1 Prescription	
	RR (95% CI)	P$_{trend}$[d]
Sex[a]		
Men	0.73 (0.64, 0.83)	<0.001
Women	0.86 (0.78, 0.94)	<0.001
P$_{interaction}$	0.029	
Age at Diagnosis/Index Date[b]**, Years**		
<50	0.99 (0.84, 1.16)	0.877
50–69	0.76 (0.68, 0.85)	<0.001
≥70	0.79 (0.69, 0.90)	<0.001
P$_{interaction}$	0.013	
Residential Ambient UVR Exposure[c]		
Low (northern Norway)	1.12 (0.67, 1.86)	0.600
Medium (central/western Norway)	0.73 (0.55, 0.95)	0.019
Highest (southern/eastern Norway)	0.82 (0.75, 0.89)	<0.001
P$_{interaction}$	0.379	

Notes: [a]Adjusted for age (by design), ever use of other drugs and residential ambient UVR exposure; [b]Adjusted for sex (by design) ever use of other drugs and residential ambient UVR; [c]Adjusted for sex and age (by design) and ever use of other drugs; [d]Trend test uses the median number of prescriptions in all three prescription categories (≤1; 2−7; ≥8).

respectively (P$_{interaction}$ 0.013). In the residential regions with medium and highest ambient UVR, ≥8 prescriptions were associated with a 27% and 18% reduced melanoma risk, respectively, while no association was found in the low UVR region (P$_{interaction}$ 0.379). Analyses using the five-level variable of residential ambient UVR exposure gave similar results to that with three-levels (not shown). We found no significant interaction with parity (P$_{interaction}$ 0.248).

Discussion

Based on data from nationwide health registries, we found that ≥8 prescriptions of antidepressants were associated with decreased melanoma risk. Analyses stratified by antidepressant classes showed a significant negative association and trend for SSRIs and mixed antidepressants, as well as a negative trend for the class of other antidepressants. For cumulative dose (DDD), a significant negative trend was found for antidepressant use overall and for SSRIs, despite the effect estimates not supporting a dose–response relationship. The negative association was significant for both sexes, age ≥50 years, in residential

regions with medium and highest ambient UVR exposure, all histological subtypes, tumors at trunk, upper and lower limb, and for localized disease at diagnosis.

Depression and mood disorders may promote cancer processes through several pathways,[11,12] though the epidemiological evidence for depression being a risk factor for cancer is not strong.[38] However, a comprehensive European case-control study identified factors of stress, traumatic events and depression as being associated with an increased risk of melanoma.[10] On the other hand, antidepressants treat depression disorders and are also found to normalize the pathway alterations that occur during depression.[11] Preclinical studies have raised the question of whether such drugs can influence cancer risk. The predominant findings indicate cancer-inhibitory actions of antidepressants, although cancer-promoting effects have also been observed.[18,19] It is worth noting though that such studies vary with regard to design and type of antidepressant. It is suggested that antidepressants may affect pathways which inhibit the malignant cell cycle and activates the immune system in ways that trigger apoptosis in

cancer cells.[18,39,40] Cancer-inhibiting actions from antidepressants, both SSRIs[20,41] and TCAs,[21] are observed for melanoma cells in vivo and in vitro.

Over the last two decades, an increasing number of observational studies have investigated the association between antidepressants and cancer, mainly focusing on breast, ovarian and colon cancer. Results from the first studies suggested a positive association, but over time, the findings predominantly indicated a negative association (mainly for SSRIs).[18] A previous review found that short-term and/or low-dose antidepressant use increased the risk of breast and ovarian cancer.[42] More recently however, a prospective cohort study, within the Nurses' Health Study (USA), found no associations for breast cancer[43] and a nationwide registry-based case-control study from Denmark found a negative association between SSRIs and epithelial ovarian cancer.[44]

To our knowledge, this is the first observational study investigating the relation between antidepressants and melanoma risk. Our results are in line with the cancer-inhibiting properties exhibited by these drugs in pre-clinical studies, including the more recent observational studies of other cancer types. The different results observed across antidepressant classes however, may be due to different action pathways inherent to each drug[45] but may also result from small groups and weakened power in stratified analyses.

We found a significant negative association for both sexes, although it seemed to be stronger for men than for women. Reproductive factors and female sex hormones are suggested to play a role in melanoma development.[34,35] A recent population-based cohort study of Norwegian women found no association between reproductive factors and melanoma risk[46] and neither did our analyses. Adjusting for number of children (for women only) did not influence the estimates. Regarding menopausal hormone use, a nationwide cohort study based on Finnish data recently reported a significant positive association between menopausal estrogen therapy and melanoma risk.[47] In our analyses, we have adjusted for other drug use, however, we were not able to separate estrogen use from other drugs.

The elevated risk observed in non-users, compared to the lowest cumulative dose (DDD 1–91), weakens the hypothesis that drug effects explain our findings. Patients with depression are less active in everyday social life,[48] which might also include activities involving UVR exposure. As far as we know, neither depression nor use of antidepressants has been associated with altered habits of outdoor activity and reduced UVR exposure. Increased use

of indoor tanning has been observed,[49,50] although such studies only involve age groups <50 years. If a decrease in social functioning among patients with depression leads to a reduction in UVR exposure, the potential difference in UVR exposure between the healthy and the depressed should be more distinct in areas with higher ambient UVR exposure, which is in line with our result. Thus, our data do not support the use of antidepressants as a preventive measure against melanoma.

In analyses by duration of use, the negative association was significant for both user categories among long-term users (>5 years), but only for the highest user category among short-term users (<5 years). Such results are in line with potential cancer-inhibiting actions induced by long-term use or a considerable short-term use of antidepressants. However, if the most depressed are those most affected socially, and represent those treated over a longer timeframe or have a higher number of prescription over a shorter timeframe, then reduced UVR exposure could be a possible explanation.

The main strengths of this study include the use of complete and high-quality data, collected from nationwide registries with mandatory reporting. This approach eliminated selection and recall bias and ensured detailed information about invasive melanoma diagnoses and drug use. Only pre-diagnostic prescriptions of antidepressants were included, and all prescriptions given the year before diagnosis/index date were removed for cases and controls, respectively. Primary non-adherence is not an issue since only information pertaining to drug dispensation and purchase by patients is recorded in the NorPD, and are more indicative of use than prescriptions alone.[51] In addition, the agreement between filled prescriptions data and self-reported antidepressant medication is reported to be good.[52]

There are also limitations when interpreting the results of this study. We had no information about individual UVR exposure, such as recreational sun exposure, sunburns (as a marker of severe acute UV exposure episodes) and indoor tanning. As the latency time between drug exposure and melanoma diagnosis is uncertain, the follow-up time might have been insufficient to reveal the true association between use of antidepressants and melanoma risk. Underlying indications for drug use and comorbidities that influence the risk of melanoma may have introduced potential confounding by indication. Although we have adjusted for use of other drug types, we cannot exclude the possibility that different indications for different antidepressant could have produced different estimates

for the drug classes. Furthermore, different indications may be related to other relevant factors (eg, obesity, socio-economic factors, hormone use, and alcohol consumption). Unfortunately, we lack information about such potentially confounding factors. In addition, differences in healthcare usage may influence our results, although adjustment for use of other drugs could act as an indicator for this. While the majority of people in Norway live in the southern/eastern regions, we cannot rule out that access to health-care and prescription practices may vary between regions.

Conclusions

The negative associations observed between antidepressant use and melanoma risk can result from at least two possible explanations: cancer-inhibiting actions induced by the drug and less UVR exposure among the most frequent users of antidepressants, compared to non-users. To investigate this further, studies with individual information about UVR exposure and indication for drug use are required.

Abbreviations

All are defined in full at their first instance in the text: ATC, anatomical therapeutic chemical; CI, confidence interval; CRN, Cancer Registry of Norway; DDD, defined daily doses; ICD-O-3, International Classification of Diseases of Oncology 3rd edition; ICD-7/10, International Classification of Diseases 7th/10th Revision; NM, nodular melanoma; NorPD, Norwegian Prescription Database; PIN, personal identification number; RR, rate ratio; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressants; UVR, ultraviolet radiation.

Ethics Approval and Informed Consent

The study is approved by the Norwegian Data Protection Authority and the Regional Committee for Medical and Health Research Ethics. The study is also approved by the national registries contributing with data; CRN, the National Registry, NorPD and the Medical Birth Registry. The linkage key for the 11-digit PINs was stored and governed by a third party unavailable to the research team. All data management and analyses were conducted on data with no individual person identified. This case-control study utilized only data from nationwide population-based registers and thus did not include a recruitment process for patients, who were not involved in neither the design nor conduct of the study. Thus, the research

question and outcome measures were not informed by any specific patient priorities, experiences or preferences. Rather, their formulation was based upon our own priorities for patient benefit and result interpretation. All results are distributed on a group level, without any possibilities for individual identification.

Data Sharing Statement

The data is available as presented in the paper. According to Norwegian legislation, our approvals to use the data for the current study do not allow us to distribute or make the data directly available to other parties.

Author Contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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Paper II

ORIGINAL RESEARCH

Use of Immunomodulating Drugs and Risk of Cutaneous Melanoma: A Nationwide Nested Case-Control Study

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Purpose: Cutaneous melanoma is among the fastest growing malignancies in Norway and ultraviolet radiation (UVR) exposure is the primary environmental risk factor. Immunomodulating drugs can increase skin photosensitivity and suppress immune responses, and by such mechanisms influence melanoma risk. We, therefore, aimed to examine the associations between use of immunomodulating drugs and melanoma risk, at a nationwide population level.

Patients and Methods: In the Cancer Registry of Norway, we identified all cases aged 18–85 with a first primary cutaneous melanoma diagnosed in 2007–2015 (n=12,106). These were matched to population controls from the Norwegian National Registry 1:10 (n=118,564), on sex and year of birth using risk set sampling. Information on prescribed drugs (2004–2015) was obtained by linkage to the Norwegian Prescription Database (NorPD). Conditional logistic regression was used to estimate rate ratios (RRs) and 95% confidence intervals (CIs) for associations between use of immunomodulating drugs (immunosuppressants and corticosteroids) and melanoma risk, adjusted for ambient UVR and other drug use.

Results: Compared with ≤1 prescription, use of ≥8 prescriptions of immunosuppressants was associated with increased risk of melanoma (RR 1.50, 95% CI 1.27, 1.77). Similar associations were found for subgroups of immunosuppressants: drugs typically prescribed to organ transplant recipients (OTRs) (RR 2.02, 95% CI 1.35, 3.03) and methotrexate (RR 1.27, 95% CI 1.04, 1.55). Similar results were found for high levels of cumulative doses and across all histological subtypes. Use of corticosteroids was not associated with melanoma risk.

Conclusion: We found a positive association between use of immunosuppressants and melanoma risk, with the highest risk seen for drugs prescribed to OTRs. Knowledge about this risk increase is important for physicians and users of these drugs, for intensified surveillance, awareness and cautious sun exposure.

Keywords: immunosuppressants, corticosteroids, melanoma, prescription drugs, pharmacoepidemiology, registry-based

Summary

The number of melanoma cases has reached historically high levels in fair-skinned populations worldwide, and is most likely caused by excessive sun exposure. However, immunomodulating drugs may influence the risk of melanoma by affecting our immune system's tumor surveillance or increasing the skin's sensitivity to sunlight. Such drugs include both immunosuppressants and systemic corticosteroids, typically prescribed to treat long-term autoimmune diseases and to prevent organ transplant

rejection. As far as we know, this is the first epidemiological study that examines the association between use of immunomodulating drugs (including immunosuppressants and systemic corticosteroids) given for any indication, and melanoma risk on a nationwide level.

Compared with non-use, users of immunosuppressive drugs had an increased risk of melanoma, with the highest risk seen for drugs designed to prevent organ transplant rejection. Use of corticosteroids was not associated with melanoma risk. The side effects of immunosuppressive drugs, including immune suppression and increased photosensitivity in the skin, are possible explanations for these results. The findings could be valuable for both physicians and users of these drugs, as they could help reduce melanoma risk by increasing surveillance and awareness, which could lead to more careful sun exposure behavior.

Introduction

Cutaneous melanoma (hereafter melanoma) is the skin cancer that causes the highest number of deaths. It is the malignancy with the most rapid growth rate in Norway, which ranks among the top 3 countries worldwide, both in terms of incidence and mortality.[1,2] The primary environmental risk factor for melanoma is ultraviolet radiation (UVR) exposure, which is estimated to be responsible for as much as three-quarters of all cases worldwide.[3] The development of melanoma, however, is a process dependent on many factors in which individual susceptibility (number of nevi and skin sensitivity to UVR[4]), previous melanoma diagnosis,[5] family history of melanoma,[6] and additional factors such as anthropometric measures,[7] hormonal factors[8,9] and alcohol consumption[10] are suggested to influence the risk.

Immunomodulating drugs comprise immunosuppressants and systemic corticosteroid hormones (hereafter corticosteroids), and are typically used for the treatment of inflammatory and autoimmune diseases, as well as to prevent the rejection of transplanted organs.[11,12] The immunosuppressive actions of these drugs have a well-documented list of side-effects and toxicities due to the non-specific nature of immunesuppression.[13,14] It is well-established that the immune system has an important role in the progression and regression of melanoma, and therefore it is likely that the development of this cancer is affected by long-term exposure to immunomodulating drugs.[15] Organ transplant recipients (OTRs) are shown to experience an increased risk of melanoma,[16–19] though such a relationship is not clear for other patient groups who use immunosuppressant drugs.[20] Methotrexate is

a commonly prescribed immunosuppressant, used to treat inflammatory and autoimmune disorders, such as rheumatoid arthritis and psoriasis. Studies indicate an association between methotrexate use and risk of melanoma and melanoma-specific mortality,[21–23] although no dose-response association has been discovered.[24] Other drugs with immunosuppressant actions, commonly used to treat these diseases, are also suggested to increase the melanoma risk.[25–27] Furthermore, the propensity of certain immunosuppressants to exacerbate UVR-induced DNA damage is theorized to be responsible for a potential elevated risk of melanoma.[28,29]

Corticosteroids, and glucocorticoids in particular, are used as part of a variety of anti-inflammatory and immunosuppressive therapies, for conditions such as rheumatoid arthritis, inflammatory bowel syndrome, psoriasis, and eczema.[13] Epidemiological studies have shown that use of glucocorticoids is associated with increased risk of non-melanoma skin cancers,[30–32] and corticosteroids are found to increase the risk of cutaneous T-cell lymphoma.[33] Epidemiological studies concerning a relationship with melanoma risk appear to be lacking. Pre-clinical studies, however, have demonstrated an ability by certain glucocorticoids (particularly dexamethasone) to inhibit human melanoma growth.[34,35]

To our knowledge, no nationwide epidemiological studies have investigated the associations between the prescribed use of immunosuppressants and corticosteroids, given for any indication, and melanoma risk. We aimed to investigate this association in a nested case-control study, employing population-based registries in Norway.

Materials and Methods

This case-control study was nested within the Norwegian National Registry, encompassing the entire adult Norwegian population during the period 2004–2015 (3.9 million people). A nested case-control design was chosen due to a principle of data minimization. Data were drawn from the Cancer Registry of Norway (CRN), the National Registry, the Norwegian Prescription Database (NorPD), and the Medical Birth Registry of Norway (Figure S1). Unique personal identification numbers (PIN) assigned to each person residing in Norway, were used to link the data across registries. Data collection procedures and study design features have been described.[36]

Selection of Cases and Controls

By law, the CRN has recorded data regarding cancer diagnoses since 1953. After 2000, >99% of melanoma

cases have been morphologically verified.[1,37] We selected all first primary melanoma cases in the CRN, which were diagnosed at the age of 18–85 in the period 2007–2015 (n=12,106). The cases were recorded according to the International Classification of Diseases (ICD) of oncology 3rd edition (ICD-O-3), and the ICD 10th Revision (C43). Tumor site was categorized as head/neck (C43.0–4), trunk (C43.5), upper limb (C43.6), lower limb (C43.7), other (C43.8) and unspecified (C43.9). The histological subtype of each tumor (recorded according to ICD-O-3) was categorized as superficial spreading melanoma (SSM; 87433), nodular melanoma (NM; 87213) or other subtypes (87423, 87443, 87453/87803/87613, and 87203 (unspecified)). The CRN records the stage of melanoma at diagnosis based on clinical and pathological information on metastasis, which is the basis for its categorization as local disease (no metastases), regional metastasis (regional lymph nodes, satellites and in transit metastases), distant metastasis (non-regional lymph node and organ metastases) and unspecified.

For each melanoma case, 10 controls were drawn at random (with replacements) from the National Registry using risk set sampling, matched on sex and year of birth (n=118,564). The controls in question had to be alive, free of any previous cancer diagnosis, and had to reside in Norway at their index date (date of diagnosis for respective case). This meant, however, that they could develop cancer afterwards. Permission to conduct the study was granted by the Norwegian Data Inspectorate, the Regional Committee for Medical and Health Research Ethics and each of the relevant registries.

Assessment of Immunomodulating Drug Use

All prescription drugs dispensed from Norwegian pharmacies have been recorded by the NorPD since 1 January 2004, except for those prescribed to institutionalized individuals.[38] Drugs are classified according to the Anatomical Therapeutic Chemical (ATC) classification system version 2017.[38] Information about the date, prescriber, patient and drug is included for each record of dispensation.[37] Information was obtained for the period 2004–2015, including the date of dispensing, ATC classification code, and the number of defined daily doses (DDD) dispensed, which is defined as the assumed average maintenance dose per day for a drug used for its main indication in adults.[39] Immunosuppressants were defined as any drug included in the ATC group L04.[40]

Due to the elevated risk of skin cancer reported for OTRs and because the most commonly prescribed immunosuppressant was methotrexate (L04AX03) (11.5%), immunosuppressant drugs commonly used by OTRs (L04AA06/10/18, L04AD01/02) and methotrexate (L04AX03) constituted two separate groups for analysis. Users of all remaining immunosuppressants constituted the category "Other drugs with immunosuppressant actions" (selective immunosuppressants (ATC codes L04AA13/21/24/27/31), tumor necrosis factor alpha inhibitors (ATC codes L04AB01/02/04/05/06), interleukin inhibitors (ATC codes L04AC03/05), and other immunosuppressive drugs (ATC codes L04AX01/02/05)).[40] Corticosteroids were defined as any drug included in the ATC group H02, and were analyzed as one group due to the many different and overlapping indications for use. The most commonly prescribed type of corticosteroid, however, was glucocorticoids (99.2%).

We quantified the number of prescriptions for each person based on their total number of filled prescriptions over the period 2004–2015. Exclusive use of immunosuppressants or corticosteroids for each individual was categorized according to the number of filled prescriptions since 2004: ≤1 prescription, 2–7 prescriptions and ≥8 prescriptions.[41] This was based on the assumption that each immunomodulating drug prescription is equivalent to 3 months of use. For methotrexate, each prescription may last up to 6 months depending on dosing regimen,[42] thus the use was categorized as ≤1 prescription, 2–3 prescriptions and ≥4 prescriptions. While repeated prescriptions indicate sustained use of a drug, a single prescription reflects non-use or very limited use due to reasons such as lack of effect, side-effects, non-compliance or insufficient follow-up within the time period of the study. We therefore chose to combine non-use and limited use in the same category. The prescriptions in question could have been filled at separate or the same dates.

Cumulative dose of overall immunosuppressants or corticosteroids was calculated based on the total number of DDDs filled for each person and was categorized as non-users (0) and users (1–365; 366–1100; 1101–1800; 1801–2900; ≥2901). For subgroup analyses, immunosuppressants prescribed to OTRs, methotrexate and "Other drugs with immunosuppressant actions", DDD were categorized as 1–365; 366–1800; ≥1801. The DDD categorizations were formed based on the assumption that immunomodulating drugs are typically prescribed for long-lasting inflammatory and/or immunosuppressive indications.[12,13] The user levels thus corresponded to <1,

1–3, 4–5, 6–8, >8 years of use, though some of these levels were collapsed in subgroup analyses due to low numbers of drug users.

All prescriptions filled within a year prior to and after individual index dates (date of diagnosis for cases and respective controls) were disregarded to reduce potential impact of reverse causation.

Covariates

There are several drugs which could influence the risk of melanoma by affecting skin photosensitivity or the mechanisms of the immune system.[43–46] Therefore, information about ever-use of cardiovascular drugs (yes/no), antidepressant drugs (yes/no), and whether >1 prescription was filled of any other prescription drug (yes/no) was drawn from the NorPD, for the period 2004–2015. We used the information about use of such drugs up to a year prior to individual index dates.[45,47]

Ambient UVR exposure decreases from southern to northern Norway. The level of ambient UVR exposure was categorized according to the region of residence for each person. This information was obtained from both the CRN (for cases) and the NorPD (for controls),[48] and was categorized into a five-level covariate: southeast coast, southeast inland, southwestern Norway, central Norway and northern Norway.[7] We also categorized this information into a three-level covariate with highest (eastern and southern Norway), medium (western and central Norway) and lowest (northern Norway) levels of UVR exposure.[49] Persons (both cases and controls) that lacked information concerning region of residence were excluded (67 cases and 3257 controls).

Parity is suggested to influence melanoma risk through female sex hormones,[50,51] or factors related to parity (socioeconomic status and sunbathing habits).[50] Therefore, we obtained individual information on number of children (up until index date) for all women from the Medical Birth Registry, and categorized it as 0, 1–3 and >3 children.

Statistical Analyses

We used conditional logistic regression to investigate the association between the use of prescribed immunosuppressant and corticosteroid drugs, and melanoma risk, estimating rate ratios (RRs) with 95% confidence intervals (CIs).[52] All analyses were adjusted for sex, year of birth and index date by design, including residential ambient UVR exposure and other drug use. Immunosuppressant

(L04) and corticosteroid (H02) drug use were also mutually adjusted. Analyses of OTR drugs and methotrexate were adjusted for corticosteroid (H02) drug use, including use of non-OTR and non-methotrexate immunosuppressive drugs, respectively. The results from the multivariable analyses are presented without adjustment for parity (for women), since this covariate had a negligible effect on the effect estimates.

A likelihood ratio test was used to test for interactions, on the multiplicative scale, between the number of immunosuppressant and corticosteroid prescriptions, and age (at index date), sex, ambient UVR exposure level (three level variable) and parity (women only). RRs were also calculated for the associations between the number of prescriptions of immunosuppressants and corticosteroids, and stratified sex, age (at index date), and residential ambient UVR exposure. Lastly, we conducted stratified analyses for tumor site, histological subtype, and clinical stage at diagnosis, in which contrast tests for heterogeneity between effect estimates were performed.[53]

The statistical software package R (version 3.5.1) was used to conduct all statistical analyses.[54] The significance level was set to 5%, and all tests were two-sided.

Results

The study sample consisted of 130,670 individuals, including 5988 male and 6118 female cases of melanoma diagnosed in the period 2007–2015, and 58,309 male and 60,255 female population controls (Table 1). Most cases and controls were ≥50 years at index date and resided in the southern and eastern regions of Norway. The majority of melanomas were located on the trunk (47%). SSM was the most frequent histological subtype (55%), and most tumors were diagnosed with no metastasis (81%). Of the study population, 1.8% had filled at least 1 immunosuppressant prescription, of which 60.5% had ≥8 prescriptions, and 9.4% were in the highest dose level. By contrast, 14.7% had filled at least 1 corticosteroid prescription, of which 16.8% had ≥8 prescriptions and 0.6% were in the highest dose level. While not depicted in Table 1, people who had filled both immunosuppressant and corticosteroid prescriptions constituted 1.5% of all study participants, and 9.7% of all immunomodulating drug users.

A 50% increased risk of melanoma was found in individuals with ≥8 prescriptions filled of any immunosuppressant drug, compared with ≤1 prescription filled (Table 2). When investigating immunosuppressant drugs prescribed to OTRs, ≥8 prescriptions was associated with

Table 1 Characteristics of Cases and Controls in the Study Sample

Characteristics	Cases (n=12,106) No. (%)	Controls (n=118,564) No. (%)
Sex		
Men	5988 (49.5)	58,309 (49.2)
Women	6118 (50.5)	60,255 (50.8)
Age at diagnosis/index date, years		
<50	3163 (26.1)	30,348 (25.6)
50–69	5616 (46.4)	55,279 (46.6)
≥70	3327 (27.5)	32,937 (27.8)
Residential ambient UVR[a] exposure		
Lowest (northern Norway)	717 (5.9)	11,906 (10.0)
Medium (central/western Norway)	1725 (14.3)	19,445 (16.4)
Highest (southern/eastern Norway)	9664 (79.8)	87,213 (73.6)
Parity (women only)		
No children	1864 (30.5)	18,994 (31.5)
1–3 children	3895 (63.7)	36,942 (61.3)
>3 children	359 (5.8)	4319 (7.2)
Tumor site		
Head/neck	1315 (10.9)	–
Trunk	5661 (46.8)	–
Upper limb	1559 (12.9)	–
Lower limb	2869 (23.7)	–
Other	52 (0.4)	–
Unspecified	650 (5.3)	–
Histological subtype		
Superficial spreading melanoma	6656 (55.0)	–
Nodular melanoma	2079 (17.2)	–
Other	3371 (27.8)	–
Clinical stage		
Local disease	9833 (81.2)	–
Regional metastasis	573 (4.7)	–
Distant metastasis	635 (5.2)	–
Unspecified	1065 (8.8)	–
Use of immunomodulating drugs [b] Overall use by number of prescriptions		
≤1	11,029 (91.1)	107,998 (91.1)
2–7	647 (5.3)	6791 (5.7)
≥8	430 (3.6)	3775 (3.2)
Use by number of prescriptions of each drug type		
≤1	11,029 (91.1)	107,998 (91.1)
I. Immunosuppressants (L04)[c]		
2–7	14 (0.1)	190 (0.1)
≥8	40 (0.3)	343 (0.3)

(Continued)

Table 1 (Continued).

Characteristics	Cases (n=12,106) No. (%)	Controls (n=118,564) No. (%)
II. Corticosteroids (H02)[d]		
2–7	612 (5.0)	6392 (5.4)
≥8	201 (1.7)	2123 (1.8)
Use by cumulative dose (DDD[e])		
0	10,283 (84.9)	100,488 (84.8)
I. Immunosuppressants overall (L04)[c]		
1–365	11 (0.1)	197 (0.2)
366–1100	23 (0.2)	189 (0.2)
1101–1800	13 (0.1)	89 (0.1)
1801–2900	6 (0.05)	96 (0.1)
≥2901	6 (0.05)	39 (0.0)
II. Corticosteroids overall (H02)[d]		
1–365	1353 (11.2)	13,793 (11.6)
366–1100	137 (1.1)	1580 (1.3)
1101–1800	43 (0.4)	342 (0.3)
1801–2900	17 (0.1)	164 (0.1)
≥2901	4 (0.05)	69 (0.0)
Ever use of other drugs		
Antidepressant drugs (N06A)[f]	1870 (15.4)	20,243 (17.1)
Cardiovascular drugs (C1–10)[g]	5840 (48.2)	56,263 (47.5)
Other drugs[h]	5450 (45.0)	52,964 (44.7)

Notes: [a]Ultraviolet radiation; [b]Does not show number of individuals dispensed both immunosuppressant and corticosteroid prescriptions (mixed use); [c]Users of immunosuppressants only; [d]Users of corticosteroids only; [e]Defined daily dose; [f]Use of antidepressants, but not cardiovascular drugs; [g]Use of cardiovascular drugs, but not antidepressants; [h]Use of all other drugs other than antidepressant or cardiovascular drugs.

a two-fold increased risk of melanoma, compared with ≤1 prescription. For methotrexate, ≥4 prescriptions was associated with a 27% increased melanoma risk, compared with ≤1 prescription. No associations were found for use of "Other drugs with immunosuppressant actions" or corticosteroids (Table 2). When analyzing the use of immunosuppressants overall by cumulative dose in DDDs, significantly elevated RRs were found for all DDD categories ≥366 (except 1801–2900), compared with 1–365 (Table 3). Analyses by drug group showed increased RRs by increasing DDD for OTR drugs in particular (RR 1.81, 95% CI 0.76, 4.30), but also for methotrexate (RR 1.47, 95% CI 0.89, 2.40), although not statistically significant. No significant associations were found for cumulative doses of corticosteroids.

Compared with ≤1 prescription, ≥8 prescriptions of immunosuppressants was associated with increased risk

Table 2 Rate Ratios (RRs) with 95% Confidence Intervals (CIs) for Number of Prescriptions of Immunomodulating Drugs and Melanoma Risk

Number of Prescriptions	No. Case/Controls	RR (95% CI)[a]
Immunosuppressants overall (L04)[b]		
≤1	11,858/116,663	1.00 (reference)
2–7	63/637	0.98 (0.75, 1.28)
≥8	185/1264	1.50 (1.27, 1.77)
OTR[c] drugs (L04AA06/10/18, L04AD01/02)[d]		
≤1	12,069/118,363	1.00 (reference)
2–7	6/50	1.11 (0.47, 2.61)
≥8	31/151	2.02 (1.35, 3.03)
Methotrexate (L04AX03)[e]		
≤1	11,950/117,270	1.00 (reference)
2–3[f]	22/267	0.79 (0.51, 1.22)
≥4[f]	134/1027	1.27 (1.04, 1.55)
Other drugs with immunosuppressant actions (L04AA13/21/24/27/31, L04AB01/02/04/05/06, L04AC03/05, L04AX01/02/05)[g]		
≤1	12,002/117,736	1.00 (reference)
2–7	27/249	1.00 (0.66, 1.50)
≥8	77/579	1.19 (0.91, 1.54)
Systemic corticosteroids (H02)[h]		
≤1	11,101/108,721	1.00 (reference)
2–7	691/6917	0.96 (0.88, 1.04)
≥8	314/2926	0.98 (0.86, 1.11)

Notes: [a]Estimated RRs with 95% CIs, due to risk-set sampling of controls, adjusted for sex and birth year (by design) and ever use of other drugs and residential ambient ultraviolet radiation exposure; [b]Additionally adjusted for number of systemic corticosteroid prescriptions; [c]Organ transplant recipient; [d]Additionally adjusted for number of non-organ transplant immunosuppressants and systemic corticosteroid prescriptions; [e]Additionally adjusted for number of non-methotrexate immunosuppressants and systemic corticosteroid prescriptions; [f]Methotrexate prescription categories compensate for comparatively longer duration of use; [g]Additionally adjusted for number of organ transplant and methotrexate immunosuppressant and systemic corticosteroid prescriptions; [h]Additionally adjusted for number of immunosuppressant prescriptions.

Table 3 Rate Ratios (RRs) with 95% Confidence Intervals (CIs) for Cumulative Defined Daily Dose (DDD) of Immunomodulating Prescription Drugs and Melanoma Risk

Cumulative Dose, DDD	No. Case/Controls	RR (95% CI)[a]
Immunosuppressants overall (L04)[b]		
0	11,837/116,436	1.14 (0.86, 1.50)
1–365	56/635	1 (Reference)
366–1100	92/657	1.61 (1.13, 2.29)
1101–1800	52/344	1.75 (1.17, 2.61)
1801–2900	38/297	1.45 (0.94, 2.25)
≥2901	31/195	1.75 (1.09, 2.80)
OTR[c] drugs (L04AA06/10/18, L04AD01/02)[d]		
0	12,067/118,352	0.79 (0.41, 1.55)
1–365	10/74	1 (Reference)
366–1800	12/71	1.36 (0.55, 3.37)
≥1801	17/67	1.81 (0.76, 4.30)
Methotrexate (L04AX03)[e]		
0	11,927/117,072	1.07 (0.78, 1.46)
1–365	45/472	1 (Reference)
366–1800	104/801	1.37 (0.94, 1.98)
≥1801	30/219	1.47 (0.89, 2.40)
Other drugs with immunosuppressant actions (L04AA13/21/24/27/31, L04AB01/02/04/05/06, L04AC03/05, L04AX01/02/05)[f]		
0	11,994/117,628	1.11 (0.77, 1.60)
1–365	34/343	1 (Reference)
366–1800	59/426	1.34 (0.85, 2.09)
≥1801	19/167	1.07 (0.59, 1.95)
Systemic corticosteroids (H02)[g]		
0	10,342/101,098	1.04 (0.98, 1.11)
1–365	1445/14,450	1 (Reference)
366–1100	203/2080	0.95 (0.81, 1.11)
1101–1800	69/569	1.11 (0.85, 1.45)
1801–2900	36/268	1.21 (0.85, 1.74)
≥2901	16/99	1.54 (0.90, 2.63)

Notes: [a]Estimated RRs with 95% CIs, due to risk-set sampling of controls, adjusted for sex and birth year (by design) and ever use of other drugs and residential ambient ultraviolet radiation exposure; [b]Additionally adjusted for cumulative corticosteroid dose; [c]Organ transplant recipient; [d]Additionally adjusted for cumulative dose of non-organ transplant immunosuppressants and systemic corticosteroids; [e]Additionally adjusted for cumulative dose of non-methotrexate immunosuppressants and systemic corticosteroids; [f]Additionally adjusted for cumulative dose of organ transplant immunosuppressants, methotrexate and systemic corticosteroids; [g]Additionally adjusted for cumulative dose of immunosuppressants.

of melanoma for both men and women ($P_{interaction} = 0.068$) (Table 4). Increased risk was also found for age groups >50 years ($P_{interaction} = 0.174$) and for residential regions with the highest and medium levels of ambient UVR exposure ($P_{interaction} = 0.105$). There were negligible differences in the results of multivariable analyses between the five-level and three-level variable of residential ambient UVR exposure (not shown). Compared with ≤1 prescription, ≥8 prescriptions of corticosteroids was associated with a 20% reduced melanoma risk in men.

Analyses stratified by residential region ($P_{interaction} = 0.064$), showed that melanoma risk within the region of medium UVR exposure increased by number of

Table 4 Rate Ratios (RRs) with 95% Confidence Intervals (CIs) for Melanoma Risk for 2–7 and ≥8 vs ≤1 Prescription of Immunosuppressants and Systemic Corticosteroids, Stratified by Sex, Age at Diagnosis/Index Date and Residential Ambient Ultraviolet Radiation (UVR) Exposure

Variables	2–7 and ≥8 Prescriptions vs ≤1 Prescription	
	Immunosuppressants (L04)	Systemic Corticosteroids (H02)
	RR (95% CI)[a]	RR (95% CI)[b]
Sex[c]		
Men		
≤1	1.00	1.00
2–7	1.25 (0.87, 1.80)	0.93 (0.82, 1.05)
≥8	1.36 (1.05, 1.77)	0.80 (0.65, 0.98)
Women		
≤1	1.00	1.00
2–7	0.79 (0.54, 1.16)	0.98 (0.88, 1.09)
≥8	1.60 (1.29, 1.98)	1.12 (0.95, 1.31)
$P_{interaction}$[f]	0.068	0.018
Age at diagnosis/index date[d], years		
<50		
≤1	1.00	1.00
2–7	0.89 (0.46, 1.72)	1.04 (0.86, 1.24)
≥8	1.16 (0.74, 1.82)	1.15 (0.77, 1.73)
50–69		
≤1	1.00	1.00
2–7	1.18 (0.83, 1.69)	0.91 (0.81, 1.04)
≥8	1.47 (1.17, 1.85)	0.98 (0.80, 1.20)
>70		
≤1	1.00	1.00
2–7	0.76 (0.46, 1.26)	0.97 (0.85, 1.11)
≥8	1.72 (1.29, 2.29)	0.94 (0.79, 1.13)
$P_{interaction}$[f]	0.174	0.405
Residential ambient UVR exposure[e]		
Low (northern Norway)		
≤1	1.00	1.00
2–7	0.14 (0.02, 1.23)	0.84 (0.46, 1.53)
≥8	0.77 (0.27, 2.18)	1.30 (0.60, 2.84)
Medium (central/ western Norway)		
≤1	1.00	1.00
2–7	1.14 (0.44, 2.90)	0.90 (0.68, 1.19)
≥8	2.36 (1.26, 4.45)	0.47 (0.27, 0.81)
Highest (southern/ eastern Norway)		
≤1	1.00	1.00

(*Continued*)

Table 4 (Continued).

Variables	2–7 and ≥8 Prescriptions vs ≤1 Prescription	
	Immunosuppressants (L04)	Systemic Corticosteroids (H02)
	RR (95% CI)[a]	RR (95% CI)[b]
2–7	1.02 (0.76, 1.37)	0.95 (0.87, 1.04)
≥8	1.52 (1.26, 1.85)	1.01 (0.87, 1.16)
$P_{interaction}$[f]	0.105	0.064

Notes: [a]Estimated RRs with 95% CIs, due to risk-set sampling of controls, *adjusted for number of corticosteroid prescriptions*; [b]Adjusted for number of immunosuppressant prescriptions; [c]Adjusted for age (by design), ever use of other drugs and residential ambient UVR exposure; [d]Adjusted for sex (by design) ever use of other drugs and residential ambient UVR; [e]Adjusted for sex and age (by design) and ever use of other drugs; [f]Interaction is analyzed on a multiplicative scale.

immunosuppressant drug prescriptions, while risk decreased by number of corticosteroid drug prescriptions. Melanoma risk also increased by number of immunosuppressant drug prescriptions within the region of highest UVR exposure, though no association was seen for corticosteroids.

Compared with ≤1 prescription, ≥8 prescriptions of immunosuppressants was associated with increased risk of melanoma located at the head/neck (RR 2.22, 95% CI 1.45, 3.40), trunk (RR 1.67, 95% CI 1.30, 2.13) and upper limb (RR 1.51, 95% CI 0.96, 2.36), though not significant for the latter (Table 5). Significantly elevated RRs were also found for ≥8 prescriptions of immunosuppressants for all histological subtypes and for local disease and regional metastasis. No association between corticosteroids and melanoma were found across tumor site, histological subtype or clinical stage. Tests for heterogeneity found no difference between the effect estimates for tumor site, histological subtype or clinical stage, for either immunosuppressants ($P_{heterogeneity}$ = 0.112, 0.676, 0.338, respectively) or corticosteroid prescriptions ($P_{heterogeneity}$ = 0.507, 0.417, 0.260, respectively) and melanoma (Table 5).

Discussion

In this population-based study, using data from nationwide health registries, we investigated associations between use of prescribed immunosuppressant and corticosteroid drugs, and melanoma risk. Users of immunosuppressant drugs had a higher risk of melanoma. This was found for both users of ≥8 prescriptions and for increasing levels of

Table 5 Rate Ratios (RRs) with 95% Confidence Intervals (CIs) for 2–7 and ≥8 vs ≤1 Prescription of Immunosuppressants and Systemic Corticosteroids, and Melanoma Risk, Stratified by Tumor Site, Histological Subtype, and Clinical Stage at Diagnosis

	2–7 and ≥8 Prescriptions vs ≤1 Prescription	
	Immunosuppressants (L04)	Systemic Corticosteroids (H02)
	RR (95% CI)[a]	RR (95% CI)[b]
Tumor site (cases/controls)		
Head/neck (1315/12,842)		
≤1	1.00	1.00
2–7	1.71 (0.90, 3.22)	0.98 (0.78, 1.24)
≥8	2.22 (1.45, 3.40)	1.17 (0.87, 1.57)
Trunk (5661/55,107)		
≤1	1.00	1.00
2–7	1.24 (0.86, 1.79)	0.95 (0.84, 1.07)
≥8	1.67 (1.30, 2.13)	0.92 (0.75, 1.12)
Upper limb (1559/15,197)		
≤1	1.00	1.00
2–7	0.31 (0.10, 0.98)	0.95 (0.76, 1.19)
≥8	1.51 (0.96, 2.36)	1.08 (0.78, 1.50)
Lower limb (2869/28,005)		
≤1	1.00	1.00
2–7	0.72 (0.40, 1.31)	0.93 (0.78, 1.10)
≥8	1.01 (0.70, 1.47)	0.96 (0.73, 1.27)
Other (52/511)		
≤1	1.00	1.00
2–7	1.04*-8 (0, NA)	1.04 (0.29, 3.68)
≥8	2.27 (0.21, 25.09)	0.37 (0.04, 3.00)
Unspecified (650/6357)		
≤1	1.00	1.00
2–7	0.96 (0.29, 3.21)	1.08 (0.77, 1.51)
≥8	1.04 (0.43, 2.49)	0.68 (0.36, 1.28)
P$_{heterogeneity}$	0.112	0.507
Histological subtype (cases/controls)		
SSM[c] (6656/64,826)		
≤1	1.00	1.00
2–7	0.90 (0.62, 1.31)	0.97 (0.87, 1.08)
≥8	1.41 (1.12, 1.76)	0.99 (0.83, 1.19)
NM[d] (2079/20,330)		
≤1	1.00	1.00
2–7	1.02 (0.56, 1.87)	0.92 (0.75, 1.12)
≥8	1.56 (1.06, 2.31)	1.10 (0.83, 1.45)
Other[e] (3371/32,863)		
≤1	1.00	1.00
2–7	1.11 (0.69, 1.78)	0.96 (0.82, 1.12)
≥8	1.67 (1.21, 2.30)	0.86 (0.68, 1.10)
P$_{heterogeneity}$	0.676	0.417

(Continued)

Table 5 (Continued).

	2–7 and ≥8 Prescriptions vs ≤1 Prescription	
	Immunosuppressants (L04)	Systemic Corticosteroids (H02)
	RR (95% CI)[a]	RR (95% CI)[b]
Clinical stage (cases/controls)		
Local disease (9833/95,842)		
≤1	1.00	1.00
2–7	0.88 (0.65, 1.19)	0.95 (0.87, 1.04)
≥8	1.50 (1.26, 1.79)	1.03 (0.90, 1.18)
Regional metastasis (573/5610)		
≤1	1.00	1.00
2–7	0.94 (0.28, 3.12)	0.92 (0.64, 1.33)
≥8	2.17 (1.04, 4.54)	0.73 (0.41, 1.31)
Distant metastasis (635/6216)		
≤1	1.00	1.00
2–7	1.27 (0.43, 3.73)	1.01 (0.71, 1.43)
≥8	1.39 (0.60, 3.20)	0.87 (0.49, 1.54)
Unspecified (1065/10,351)		
≤1	1.00	1.00
2–7	1.99 (0.95, 4.15)	1.11 (0.81, 1.54)
≥8	0.48 (0.12, 2.03)	0.58 (0.30, 1.13)
P$_{heterogeneity}$	0.338	0.260

Notes: [a]Estimated RRs with 95% CIs, due to risk-set sampling of controls, adjusted for sex and age (by design), number of corticosteroid prescriptions, ever use of other drugs, and residential ambient ultraviolet radiation exposure; [b]Adjusted for sex and age (by design), number of immunosuppressant prescriptions, ever use of other drugs, and residential ambient ultraviolet radiation exposure; [c]Superficial spreading melanoma; [d]Nodular melanoma; [e]Includes all other histological types.

cumulative DDDs, with the highest risk observed for users of immunosuppressants prescribed for OTRs. Positive associations between melanoma risk and ≥8 prescriptions of immunosuppressants were found for all histological subtypes. No associations were found for users of corticosteroids, apart from a decreased risk of melanoma in men and for users residing in the region with medium UVR exposure.

The results from pre-clinical studies concerning the association between immunosuppressants and melanoma indicate a prevailing growth inhibition of melanoma cells, primarily through pro-apoptotic mechanisms.[55–57] Previous epidemiological studies concerning this association are primarily based on patient-cohorts, which overwhelmingly show significantly increased melanoma risk in OTRs, compared with

non-OTRs.[16–18,58] Immunosuppressants used by patient groups with conditions such as inflammatory bowel disease or psoriasis are also associated with increased melanoma risk,[26] particularly the use of methotrexate.[21,23,59,60]

As opposed to cohorts of patients, this population-based register study examined the use of immunosuppressants among melanoma cases and their controls on a nationwide basis. Although we were able to separately examine the use of drugs commonly prescribed to OTRs and for methotrexate alone, we did not have sufficient power to analyze individual drugs, or indeed by ATC 4th levels. The increased melanoma risk found with use of immunosuppressant drugs prescribed to OTRs is in line with results from previous studies and supports long-term and/or high intensity drug use of this type as a risk factor for melanoma. For methotrexate use, melanoma risk increased for users of ≥8 prescriptions, which is also in line with previous observations.[21–23] However, no association with melanoma was found for "Other drugs with immunosuppressant actions", and we were not able to clarify the indeterminate findings in previous studies for these drugs.[61–63]

We found that use of immunosuppressants increased the risk of all histological subtypes of melanoma, which could be indicative of a common mechanism of effect. A lack of heterogeneity between these effect estimates underscores this. A significantly increased risk of melanoma was found for the head/neck and trunk locations. However, a lack of heterogeneity suggested that there was no difference between the effect estimates of each location. On the other hand, the head/neck is considered more chronically exposed to UVR, while exposure at the trunk is typically repeatedly intermittent. This might be indicative of an interaction between UVR radiation and immunosuppressants with photosensitizing potential.[28] Moreover, the elevated melanoma risk in immunosuppressant users was found in regions with medium and the highest levels of ambient UVR exposure, which could support a photosensitizing effect of the drugs.[48] However, low power and a lack of individual UVR exposure metrics may be the reason for not seeing a similar effect in the Northern region; this might have likewise prevented an interaction with region of residence. There were no sex-specific differences observed. The increased risk was found for users of immunosuppressants in age groups >50 years, though low power due to a small sample size may be why a similarly increased melanoma risk was not observed in users <50 years of age, or why an interaction with immunosuppressant drug use was not found.

We found no indication of a relation between melanoma risk and use of corticosteroids, although a reduced risk was seen in men and in the region with medium UVR exposure. Previous epidemiological studies reported that use of glucocorticoids in particular is associated with risk of other cutaneous malignancies,[30–33] while this relationship seems not to be examined for melanoma. The prevailing consensus from pre-clinical studies, however, indicates an anti-proliferative effect of corticosteroids, through suppression of angiogenesis and growth of melanoma cells *in vivo*.[34,35,64–66]

A major strength of our study is that it is based on complete and high-quality registers with nationwide coverage and mandatory reporting, providing detailed information regarding melanoma diagnoses and pre-diagnostic drug use. The nature of the study precludes the influence of recall bias. An attempt to control for reverse causation bias was made by excluding prescriptions received in the last year before index date. The impact of primary non-adherence is limited by the fact that only information on drugs actually dispensed from pharmacies to patients is recorded in the NorPD, which is more indicative of use than databases including all drugs prescribed by physicians.[67]

The study also has weaknesses to consider while interpreting the results. We had no information concerning individual UVR exposure, such as behaviors related to recreational sun exposure, indoor tanning and sunburns, which might, apart from causing melanoma in their own right, interact with drugs with photosensitizing effects. For example, men who use glucocorticoids may be less exposed to sunlight and thus have a lower risk of melanoma, due to behavioral adaptations associated with an underlying disease. Regarding the increased melanoma risk for users of immunosuppressants, a reduction in outdoor activity among patients with conditions such as rheumatoid arthritis, would reduce rather than increase the risk estimates in our study. Additionally, a lack of power due to small sample sizes may have prevented the detection of significant interactions between number of prescriptions and certain covariates, hence the often wide CIs.

Immunosuppressant drug therapy often consists of several different drugs given together as part of a collective drug regimen. In addition, the number and combinations of these drugs may change over time.[11,12,68] This makes it challenging to narrow the risk of disease down to the influence of one specific drug, as an increased risk of melanoma may be due to the cumulative effect of the entire immunosuppressive drug regimen. Patient characteristics such as

indications for drug use could also be sources of residual confounding, as the risk of melanoma may be modified by the underlying conditions for which these drugs are prescribed, including autoimmune and inflammatory diseases. Moreover, 3% of controls were excluded due to lack of residential information, which was sourced by NorPD. Individuals who lacked this information would by definition consist of those who did not receive any prescription drugs. Thus, the exclusion may have introduced a certain degree of selection bias. They could, for example, represent exceptionally healthy people or long-term inpatients.

Furthermore, there were not enough users across the different drugs to properly differentiate and examine the type and severity of these wide-ranging indications. This may have introduced potential confounding by indication, especially when considering that autoimmune skin disorders (e.g. psoriasis) are treated with high-dose phototherapy which may increase the risk of melanoma.[69] Different indications can also be related to other factors suggested to influence melanoma risk (e.g. obesity, socioeconomic factors, hormones and alcohol use).[5–10] With an unknown latency time for melanoma, the follow-up time from start of drug exposure to index date may be insufficient to establish a true association.

Finally, as most people in Norway reside in the southern and eastern regions of the country, we cannot discount the possibility that intra-regional differences regarding prescription practices and access to healthcare have influenced the results.[70] However, adjusting for other drug use could act as a proxy in this regard, and we have also adjusted for residential region.

Conclusions

To our knowledge, this is the first nationwide register-based study examining the association between the use of immunomodulating drugs (immunosuppressants and corticosteroids), given for any indication, and melanoma risk. A positive association was found for higher doses of immunosuppressants. The study particularly supports drugs prescribed to OTRs, as an important risk factor for melanoma, and a positive association was also found for methotrexate. Use of corticosteroids seems not to increase the risk of melanoma. Our findings suggest that users of immunosuppressant drug types comprise a notable risk group for melanoma, and so should in addition to regular skin checkups, pursue a more cautious approach to sun exposure.

Abbreviations

All are defined in full at their first instance in the text: ATC, anatomical therapeutic chemical; CI, confidence interval; CRN, Cancer Registry of Norway; DDD, defined daily doses; ICD-O-3, International Classification of Diseases of Oncology 3rd edition; ICD-10, International Classification of Diseases 10th Revision; NM, nodular melanoma; NorPD, Norwegian Prescription Database; OTR, organ transplant recipient; PIN, personal identification number; RR, rate ratio; SSM, superficial spreading melanoma; UVR, ultraviolet radiation.

Data Sharing Statement

The data are available as presented in the paper. According to Norwegian legislation, our approvals to use the data for the current study do not allow us to distribute or make the data directly available to other parties.

Ethics Approval

The study is approved by the Norwegian Data Protection Authority and the Regional Committee for Medical and Health Research Ethics. The study is also approved by the national registries contributing with data; CRN, the National Registry, NorPD and the Medical Birth Registry. The linkage key for the 11-digit PINs was stored and governed by a third party unavailable to the research team. All data management and analyses were conducted on data with no individual person identified. This case-control study utilized only data from nationwide population-based registers and thus did not include a recruitment process for patients, who were not involved in either the design or conduct of the study. Thus, the research question and outcome measures were not informed by any specific patient priorities, experiences or preferences. Rather, their formulation was based upon our own priorities for patient benefit and result interpretation. All results are distributed on a group level, without any possibilities for individual identification.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

Øystein Karlstad participates in two Post-Authorization Safety Studies (PASS) unrelated to the submitted work. The studies are on an antidiabetic drug and an anti-psoriasis drug and have been imposed by the European Medicines Agency (EMA). The studies are funded by the marketing authorization holders (Novo Nordisk and Leo Pharma) and are conducted according to the EnCePP Code of Conduct for scientific independence and transparency. The authors report no other potential conflicts of interest related to this work.

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