Menopausal hormone therapy and risk of melanoma: do estrogens and progestins have a different role?

Journal:	*International Journal of Cancer*
Manuscript ID	IJC-17-0678.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Botteri, Edoardo; Oslo Universitetssykehus, National Advisory Unit for Women's Health; Kreftregisteret Støer, Nathalie C.; Oslo Universitetssykehus, National Advisory Unit for Women's Health Sakshaug, Solveig; Nasjonalt folkehelseinstitutt Graff-Iversen, Sidsel; Nasjonalt folkehelseinstitutt Vangen, Siri; Oslo Universitetssykehus Hofvind, Solveig; Cancer Registry of Norway, ; University College of Oslo, Health Science Ursin, Giske; Kreftregisteret Weiderpass, Elisabete; Kreftregisteret
Key Words:	skin malignant melanoma, hormone therapy, menopause, estrogen, progestin

Article type

Original article

Title

Menopausal hormone therapy and risk of melanoma: do estrogens and progestins have a different role?

Authors

E. Botteri[1,2†*], N.C. Støer[1†], S. Sakshaug[3], S. Graff-Iversen[4], S. Vangen[1,5], S. Hofvind[6,7], G. Ursin[8,9,10], E. Weiderpass[11,12,13,14]

Affiliations

1. National Advisory Unit for Women's Health, Women's Clinic, Oslo University Hospital, Oslo, Norway
2. Department of Bowel Cancer Screening, Cancer Registry of Norway, Oslo University Hospital, Oslo, Norway.
3. Department of Pharmacoepidemiology, Norwegian Institute of Public Health, Oslo, Norway.
4. Department of Non-Communicable Diseases, Norwegian Institute of Public Health, Oslo, Norway.
5. Institute of Clinical Medicine, University of Oslo, Norway
6. Oslo and Akershus University College of Applied Sciences, Faculty of Health Science, Oslo, Norway
7. Department of Mammography Screening, Cancer Registry of Norway, Oslo University Hospital, Oslo, Norway
8. Cancer Registry of Norway, Oslo University Hospital, Oslo, Norway
9. Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway
10. Department of Preventive Medicine, University of Southern California, Los Angeles, CA.
11. Department of Research, Cancer Registry of Norway, Oslo University Hospital, Oslo, Norway
12. Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, the Arctic University of Norway, Tromsø, Norway.
13. Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden.
14. Department of Genetic Epidemiology, Folkhälsan Research Center, Helsinki, Finland

***Full address for correspondence**

Edoardo Botteri, Cancer Registry of Norway, Post box 5313 Majorstuen 0304 Oslo, Norway.

Tel: +47 22 92 87 80. E-mail: Edoardo.Botteri@kreftregisteret.no

†Contributed equally to the paper

Novelty and Impact Statements

This study, based on the linkage of national registries, is the largest ever published on the role of menopausal hormone therapy in melanoma risk. We found that use of estrogen-only therapy was associated with an increase of melanoma risk, while the combination of estrogens and progestins was not. Our results suggest that estrogens and progestins might affect the risk of melanoma in opposite ways.

Abstract

The association between use of menopausal hormone therapy (HT) and occurrence of skin malignant melanoma (SMM) is controversial. We investigated the issue in a nationwide cohort of 684,696 Norwegian women, aged 45-79 years, followed from 2004-2008. The study was based on linkage between Norwegian population registries. Multivariable Poisson regression models were used to estimate the effect of HT use, different HT types, routes of administration, and doses of estrogen and progestin on the risk of SMM. During the median follow-up of 4.8 years, 178,307 (26%) women used HT, and 1476 incident SMM cases were identified. Current use of HT was associated with increased risk of SMM (rate ratio (RR) = 1.19; 95% confidence interval (CI) 1.03-1.37). Plain estrogen therapy was associated with an increased risk of SMM (RR 1.45; 95% CI 1.21-1.73), both for oral (RR 1.45; 95% CI 1.09-1.93) and vaginal (RR 1.44; 95% CI 1.14-1.84) formulations, while combined estrogen and progestin therapy (EPT) was not (RR 0.91; 95% CI 0.70-1.19). We performed a dose-response analysis of estrogen and progestin in women using tablets, and found that use of estrogens was associated with increased risk (RR 1.24; 95% CI 1.00-1.53 per 1 mg/day) and use of progestins with decreased risk (RR 0.71; 95% CI 0.57-0.89 per 10 mg/month) of SMM. In conclusion, estrogens were associated with increased risk of SMM, while combinations of estrogens and progestins were not. Our results suggest that estrogens and progestins might affect the risk of SMM in opposite ways.

Keywords: skin malignant melanoma, hormone therapy, menopause, estrogen, progestin

Introduction

The incidence rate of skin malignant melanoma (SMM) has been steadily increasing globally in the last decades. It was estimated that SMM accounted for 232,000 new cancer

cases and 55,000 deaths worldwide in 2012 [1]. Known risk factors for SMM include sun exposure, fair complexion, number of nevi and freckles and family history of SMM [2-4]. Hormonal factors, both endogenous and exogenous, have been suggested to be associated with the risk of SMM. A meta-analysis of epidemiological studies published up to 2009 showed that the risk of SMM was positively associated with age at first pregnancy and inversely associated with parity [5]. A 2011 large French cohort study suggested a reduced SMM risk associated with decreased exposure to endogenous ovarian hormones, namely late age at menarche, early natural menopause, and shorter ovulatory life [6]. The association between hormone therapy, both oral contraceptives (OC) and menopausal hormone therapy (HT), and SMM is controversial. The above-mentioned meta-analysis reported no association of OC or HT use with SMM risk. However, a 2009 large case-control study from the Netherlands reported a strong detrimental effect of OC and plain estrogen HT on SMM risk [7].

We conducted a nationwide cohort study, based on the linkage of population-based registries, to estimate the association between HT use and the risk of SMM. We examined the association between different types, routes of administration and doses of HT on the risk of SMM. In particular, we focused on the different effects of estrogen and the combination of estrogen and progestin on the risk of SMM. Information on age, education, sun exposure, parity, age at first birth, marital status, and use of antihypertensives, antidiabetics, statins and thyroid therapy was used to adjust all the risk estimates.

Materials and methods

Study population

The cohort is described in detail elsewhere [8]. In short, an 11-digit unique personal identification number allowed linkage of different population-based registries. Statistics Norway and the Population Registry provided information on date of birth, emigration and death, parity, county of residence, age at first birth and education level. Redeemed prescriptions were collected from the Norwegian Prescription Database and cancer data from the Cancer Registry of Norway. The study was approved by the regional ethics committee in the South East region of Norway, and concession to data linkage was granted by the Norwegian Data Protection Authority.

We included all women born between 1925 and 1959 who were alive and residing in Norway as of January 1, 2004 (aged 45-79), *n*=800,948 (Figure 1). Women with a cancer diagnosis before 2004 (*n* = 52,074) or who emigrated, experienced cancer or died within the first three months of follow-up (*n*= 2442) were excluded to ensure a minimum latency period of possible HT use. Women with prescribed sex hormones other than HT i.e. contraceptives androgens, progestogens, female hormones in combination with androgens, gonadotropins, antiandrogens and other sex hormones were excluded in order to avoid misclassification (*n*=33,299). Finally, *n*=28,430 women with only one HT-prescription during follow-up were excluded as we assumed that such a short exposure is unlikely to affect SMM risk. Additional 7 women were excluded due to erroneous date of first birth. This left 684,696 women who were followed until December 31, 2008, any cancer diagnosis, emigration or death, whichever occurred first.

[Figure 1 about here]

HT use and outcome

Data on HT use was collected from the Norwegian Prescription Database by retrieving all prescriptions of sex hormones in the anatomical therapeutic chemical (ATC)-groups G03C (estrogen) and G03F (estrogens and progestins in combination), redeemed from 2004-2008. The Norwegian Prescription Database contains detailed individual level information about all redeemed prescriptions from 2004 and onwards for the entire Norwegian population, and registration is mandatory by law. Total number of treatment days was calculated for each dispensed drug by multiplying package size by number of packages prescribed regarding the dosing intervals. For women with gaps between prescriptions, gaps shorter than 4 months were considered as continuous use, whereas longer gaps were assumed to be a stop in use with eventual re-uptake. According to their dispensed products, women were categorised as estrogen therapy (ET), tibolone (a synthetic steroid with estrogenic, progestagenic and androgenic properties) or combined estrogen-progestin therapy (EPT) users. This categorization and further categorizations according to type of combined regimen and route of administration were based on current users only. The women changing from one group to another with a gap shorter than 4 months were defined as mixed users. All EPT formulations include estradiol and norethisterone acetate as use of other progestin preparations are almost non-existent in Norway.

All women contributed person-years at risk as a non-user, current user and/or past user (Figure 2). A past-user was defined as women with more than 4 months since their last estimated use of HT. Person-years at risk within a particular category was calculated from start of study, or the date they entered the category until SMM or censoring, or the date they

moved into another category. Person-year at risk as past-user was calculated from the date the estimated duration of HT-ended.

[Figure 2 about here]

The outcome was incident SMM (International Classification of Disease Seventh Revision code 190). Non-skin melanomas and non-melanoma skin cancers were not analysed as SMM, but as censoring events.

Sun exposure

County-level information on sun exposure was extracted from the plots reported in Medhaug et al. [9]. Sun exposure was expressed as the yearly sum of erythemally weighted UV radiation (ERY), a scaled version of the UV index [10], and was available from 1957 to 2005 in 16 of the 19 Norwegian counties. ERY was quite stable within each county, and we chose the value referring to the midpoint, i.e. 1980. Sun exposure from the counties of Oslo, Oppland and Aust-Agder were not reported in the paper, so they were estimated using the mean of the exposures in the neighbouring counties.

Statistical analysis

Incidence rate ratios (RR) with 95% confidence intervals (CI) were estimated by Poisson regression. The number of incident SMM was analysed as a log-linear function of person-years at risk, HT use and adjusting covariates. Women were censored at death, emigration, other cancer diagnosis, or at end of follow-up (December 31, 2008), whichever occurred first.

We adjusted all estimates for age in years calculated at the beginning of each exposure segment, number of births (0, 1, 2, >2), age at first birth categorized in tertiles, highest level of education (elementary, high-school, university or research level, or missing), marital status

(single, married/partnered, widow or divorced/separated) and use of diabetes medication (A10), antihypertensive drugs (C02, C03, C07-C09), statins (C10) and thyroid therapy (H03) as ever-use during follow-up. In addition, all models were adjusted by county-level sun exposure, categorized as low (230-300 kiloJoule per square meter (kJ/m^2)), medium (320-360 kJ/m^2), high (370-400 kJ/m^2) or missing.

The reference group in all analyses was non-use of HT except in an additional analysis to test for a statistically significant differential effect of ET and EPT use, where ET use was used as reference category. When analysing the association of HT with a specific histological subtype (e.g. superficial spreading melanoma), only that specific subtype was analysed as events and all other SMM were censored at the date of diagnosis. Dose-response effect of estrogen and progestin use was estimated by limiting the analysis to current users of ET and EPT tablets (oral administration) and non-users only, and retrieving the oral dose of estrogen and progestin from each prescription. The dose in non-users was set to zero. The dose of estrogen and the dose of progestin were entered in a multivariable Poisson regression model as two separate continuous variables, and were therefore mutually adjusted. Since ET and EPT users might be different, we repeated the analysis in ET users only and EPT users only, as a sensitivity analysis. We did not have information on menopausal status, and all analyses were therefore repeated for women above 55 years of age at inclusion to exclude most pre- and peri-menopausal women. All tests were two-sided. Statistical analyses were performed independently by EB, using SAS 9.4 software (SAS Institute, Cary, NC) and NCS, using R 3.3.1 software (http://cran.r-project.org/).

Results

We followed 684,696 Norwegian women with no previous history of cancer from 2004 to 2008. During the median follow-up of 4.8 years, 178,307 (26%) used HT and 1476 women had a diagnosis of SMM. Table 1 displays characteristics of the study population. As the cohort is very large, all differences are significant and we only mention the most important. HT users were more educated, more likely to be married or partnered, less likely to be nulliparous and more likely to have 2 children compared to HT non-users. HT users were more likely to use anti-hypertensives and thyroid therapies compared to HT non-users. ET users were older, resided in areas with less sun exposure and had more children compared to EPT and tibolone users.

[Table 1 about here]

Current use of HT was associated with an increased risk of SMM as compared to non-use (incidence rate ratio (RR) 1.19; 95% confidence interval (CI) 1.03-1.37; Table 2), while past use was not. ET use was associated with increased risk of SMM (RR 1.45, 95% 1.21-1.73), while EPT was not (RR 0.91; 95% CI 0.70-1.19). However, there was some evidence, based on 13 SMM cases exposed only, indicating that sequential EPT was associated with an increased risk of SMM (RR 1.70, 95% 0.98-2.94). When analysing the different routes of administration, both vaginal (RR 1.44; 95% CI 1.14-1.84) and oral (RR 1.45; 95% 1.09-1.93) forms of ET increased the risk of SMM. The ET and EPT associations were significantly different in all users (p-value: 0.0036) and in oral users only (p-value: 0.016).

[Table 2 about here]

To avoid a possible confounding effect of menopausal status, unknown in our cohort, we repeated the analysis in women of 55 years or older at cohort entry (Table 2). The results

remained mostly unchanged, except that the association between tibolone and SMM risk was now positive (RR 1.88; 95% CI 1.17-3.01; based on 18 SMM exposed cases only).

ET and EPT effect on SMM according to age, education level, county-level sun exposure, and age at first birth was reported in Table 3. ET was associated with SMM risk in all age groups and education levels. ET was not associated with the risk of SMM in women living in low sun exposure areas (RR=1.03; 95% CI 0.64-1.66). EPT was not associated with an increased risk of SSM in any of the subgroups.

[Table 3 about here]

The effect of use of oral estrogens and oral progestins were analysed in a mutually adjusted, dose-response model. Each 1 mg/day increase in oral estrogens was associated with a 24% increase in SMM risk (RR 1.24; 95% CI 1.00-1.53). Progestins showed the opposite effect, with a 29% decrease in risk for each 10 mg/month increase (RR 0.71; 95% CI 0.57-0.89). After the exclusion of EPT users, the RR associated to each 1 mg/day increase in oral estrogens was 1.23 (95% CI 0.98-1.54). After the exclusion of ET users, the RR associated to each 1 mg/day increase in oral estrogens was 1.41 (95% CI 0.82-2.44), and the RR associated to each 10 mg/month increase in oral progestins was 0.65 (95% CI 0.42-1.01). The p-value for interaction between estrogen and progestin dose was 0.10 among all ET and EPT users and 0.08 in EPT users. There were no evidence of interactions between estrogen dose and sun exposure (p-value: 0.78) or age (p-value: 0.47), or between progestin dose and sun exposure (p-value: 0.48) or age (p-value: 0.97) in all ET and EPT users or when restricting to ET or EPT users.

Stratified by histological subtypes superficial, lentigo and nodular, ET was associated with superficial SMM (RR 1.37; 95% CI 1.06-1.77). There was some indication that ET was

associated with increased risk of lentigo maligna and nodular SMM as well, but the number of exposed cases was low (results not shown).

Discussion

In this nationwide cohort study of 684,696 Norwegian women, we investigated the association between use of menopausal HT and the risk of SMM. We found an increased risk of SMM associated with the use of estrogens formulations (tablets and vaginal forms). Combined estrogen-progestin HT was not associated with an increased risk of SMM. When we analysed the effect of estrogens and progestins, we found that increasing oral doses of estrogens was associated with an increased risk of SMM, while increasing oral doses of progestins was associated with a decreased risk of SMM.

Incidence of SMM is higher among women than men between the age of 20 and 45 years, but an opposite trend is observed after the age of 50, and the female reproductive life cycle has been suggested to explain this phenomenon [11]. Several epidemiological studies investigated the possible effect of endogenous sex hormones on SMM, and it was shown that the risk of SMM decreases with number of pregnancies, with older age at menarche, with younger age at first birth and with irregular menstrual cycles, early natural menopause, and shorter ovulatory life [5-6]. The impact of exogenous sex hormones on the risk of SMM was also investigated in several epidemiological studies, still it remains uncertain. A meta-analysis of 19 cases-control studies and 6 cohort studies published up to 2009 reported no association of both OC and HT with SMM risk [5]. In particular, the authors reported a pooled relative risk, based on 7 case-control studies and 3 cohort studies, of 1.16 (95% CI 0.93-1.44) for ever versus never use of HT. No separate estimates for ET and EPT were reported in the meta-analysis, as information was not available from the original individual studies. Our estimates of ever use and current use of HT versus non-use of HT were similar to that of the meta-analysis (RR 1.12 and 1.19, respectively). We hypothesized that the lack of a clear and strong effect of HT - analysed as a whole treatment

group – on SMM risk might be due to the fact that estrogens and progestins exert opposite effects on SMM growth and progression, as it was also suggested in some in-vitro studies [12-16]. In support of this hypothesis, when we stratified the analysis by type of HT (ET versus EPT), we observed a increased risk of SMM by use of ET and a lack of effect in the case of EPT. In further support to our hypothesis, the dose-response analysis on hormones taken orally seemed to confirm the possible detrimental effect of estrogens and the protective effect of progestins on SMM risk. In agreement with our results, Koomen et al. reported, in a case-control study including 778 SMM cases, an increase in SMM risk in women who used ET for more than 6 months compared to never users (OR 2.08; 95% CI 1.37-3.14) [7], while data on EPT were not available. On the other hand, Tang et al. reported no association of ET and EPT with SMM risk in the Women's Health Initiative trials, however the evidence was based on 95 SMM cases only [17].

The association between sex hormones and the risk of SMM is biologically plausible, but the mechanisms through which they exert their effect are still largely unknown. Some in-vitro experiments suggested that estrogens might increase the proliferation of melanocytes and melanoma cells while progesterone might act as an anti-proliferative and pro-apoptotic agent, counteracting the stimulatory effects of estrogens [12-16]. Progesterone was therefore suggested to potentially act as a new anti-cancer agent for melanoma treatment [14]. Melanocytic lesions, including SMM, are positive for both estrogen and progesterone receptors [12,18,19]. Estrogen receptor beta (ERβ) is the predominant estrogen receptor in melanoma cells. Its expression was reported to decrease with increasing depth of invasion and progression and therefore ERβ was hypothesized to have an antitumor activity in SMM [20-22]. ERβ has also been considered as a possible molecular target for melanoma treatment. A meta-analysis of nine randomized clinical

trials comparing chemotherapy with Tamoxifen, which is an estrogen antagonist, to chemotherapy alone in metastatic SMM patients showed that chemotherapy with Tamoxifen improved overall and partial response, especially in women, but did not improve mortality in 1 year [23].

We found that current use of ET was associated with an increase in SMM risk, even in women using vaginal formulations. Despite their low estrogen doses, vaginal preparations can increase the estrogen levels in serum, and therefore may have a systemic effect [24-25]. However, women using vaginal ET were different from HT non-users (e.g. they were 3 years older on average as compared to HT non-users) and the estimated impact of vaginal ET on SMM risk was possibly biased from residual confounding by uncontrolled factors. Moreover, a significant number of vaginal ET users might have used oral ET before 2004, and this possibly led to an overestimation of the effect of vaginal HT on SMM risk in our analysis.

In women of 55 years or older tibolone users had an almost twofold increased risk of SMM. However, our result was based on 18 tibolone exposed SMM cases only. There was some associations, although weak, that sequential EPT was associated with an increased risk of SMM while continuous EPT was not. One could hypothesize that this discrepancy was due to the smaller doses and number of days per month of progestins in the sequential compared to continuous EPT formulations, or that cyclic stimulation is a risk factor per se.

This is the largest study on the association between HT use and SMM risk, and its main originality is given by the evaluation of a potentially different effect of ET and EPT on the risk of SMM. The registry linkages ensured detailed information on exposure of HT, including types and product information including dose of HT, and there was no recall bias as to HT used. We could retrieve information on important risk factors of SMM, such as parity and level of

education [26]. There is however a number of limitations. Although this is a large cohort, the follow-up is relatively short and the statistical power might be inadequate in some subgroups. In addition, we were not able to adjust for sun exposure on an individual level, but only for sun exposure on a group level based on UV estimates according to the place of residency. This county-level sun exposure was strongly associated with SMM incidence (data not shown). Use of solarium is an establish risk factor for melanoma, as demonstrated in prospective cohort studies in Norway [27] and elsewhere [28]. Women with a positive attitude towards HT use are more likely to use solarium compared to women refusing to use HT, and this might have resulted in an overestimation of the effect of HT on SMM incidence [29]. Although our analysis might have not been adequately adjusted for sun exposure, there is no reason to suppose that sun exposure differed in ET and EPT users, thus our result on the different effect of ET and EPT on SMM risk was not likely biased. Unfortunately, we could not adjust for the complexion phenotype and family history of SMM. We do have information on ethnicity, which could have been used as a proxy for complexion, however only 1.7% of the women in our cohort was non-Norwegian, and for this reason we decided not to use this information. We lack information on smoking and height, which are two other risk factors for SMM. It is not reasonable to believe that height in itself affects HT use, however smoking may indirectly influence HT use as smokers tend to be less concerned about their health. In addition, our estimates could be affected by the healthy user bias: it is possible that HT users were more concerned about their health than non-users and, for example, underwent more skin examinations and removals of precancerous lesions. This could have led to an underestimation of the effect of ET on SMM risk. As a proxy for the general health, we adjusted all analyses for use of other available medications: antihypertensives, antidiabetics, statins and thyroid therapy. We also chose to censor non-melanoma skin cancer

cases as their behavior, especially behaviors related to melanoma risk, such as sun exposure and awareness towards skin cancer, changes after such a diagnosis. Finally, we did not have information on menopausal status. Nonetheless, the sensitivity analysis excluding women below age 55 years to avoid a possible confounding effect of menopausal status, confirmed the results obtained in the main analysis.

In conclusion, estrogen menopausal HT was associated with an increased risk of SMM, while the combined estrogen-progestin HT was not. Increasing oral dose of estrogens was associated with an increased risk of SMM while for oral progestins the risk of SMM decreased with increasing dose. There is a need for more studies for understanding the effects of sex hormones on SMM, including types of hormones and types of regimens.

References

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359-E386.
2. Gandini S, Sera F, Cattaruzza MS, et al. Meta-analysis of risk factors for cutaneous melanoma: I. Common and atypical naevi. Eur J Cancer 2005; 41:28-44.
3. Gandini S, Sera F, Cattaruzza MS, et al. Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. Eur J Cancer 2005; 41: 45-60.
4. Gandini S, Sera F, Cattaruzza MS, et al. Meta-analysis of risk factors for cutaneous melanoma: III. Family history, actinic damage and phenotypic factors. Eur J Cancer 2005; 41: 2040-2059.

5. Gandini S, Iodice S, Koomen E, Di Pietro A, Sera F, Caini S. Hormonal and reproductive factors in relation to melanoma in women: current review and meta-analysis. Eur J Cancer 2011; 47: 2607-2617.
6. Kvaskoff M, Bijon A, Mesrine S, Boutron-Ruault MC, Clavel-Chapelon F. Cutaneous melanoma and endogenous hormonal factors: a large French prospective study. Am J Epidemiol 2011; 173: 1192-1202.
7. Koomen ER, Joosse A, Herings RM, Casparie MK, Guchelaar HJ, Nijsten T. Estrogens, oral contraceptives and hormonal replacement therapy increase the incidence of cutaneous melanoma: a population-based case-control study. Ann Oncol 2009; 20: 358-364.
8. Román M, Sakshaug S, Graff-Iversen S, et al. Postmenopausal hormone therapy and the risk of breast cancer in Norway. Int J Cancer 2016; 138: 584-593.
9. Medhaug I, Olseth JA, and Reuder J. UV radiation and skin cancer in Norway. J Photochem Photobiol B 2009; 96: 232-241.
10. Moshammer H, Simic S and Haluza D. UV "Indices"—What Do They Indicate? Int J Environ Res Public Health 2016; 13: 1041.
11. Armstrong B and English D. Cutaneous malignant melanoma, in Cancer Epidemiology and Prevention, Schottenfield D and Fraumeni JF, Editors. New York: Oxford University Press: 1996, p1282-1312.
12. Shchelkunova TA and Morozov I. Progestins and carcinogenesis. Molecular Biology 2016; 50: 7-21.
13. Wiedemann C, Nägele U, Schramm G, Berking C. Inhibitory effects of progestogens on the estrogen stimulation of melanocytes in vitro. Contraception 2009; 80:292-298.

14. Leder DC, Brown JR, Ramaraj P. In-vitro rescue and recovery studies of human melanoma (BLM) cell growth, adhesion and migration functions after treatment with progesterone. Int J Clin Exp Med 2015; 8:12275-12285.
15. Ramaraj P, Cox JL. In vitro effect of progesterone on human melanoma (BLM) cell growth. Int J Clin Exp Med 2014; 7:3941-3953.
16. Fang X, Zhang X, Zhou M, Li J. Effects of progesterone on the growth regulation in classical progesterone receptor-negative malignant melanoma cells. J Huazhong Univ Sci Technolog Med Sci 2010; 30:231-234.
17. Tang JY, Spaunhurst KM, Chlebowski RT, et al. Menopausal hormone therapy and risks of melanoma and nonmelanoma skin cancers: women's health initiative randomized trials. J Natl Cancer Inst 2011; 103:1469-1475.
18. Ohata C, Tadokoro T, Itami S. Expression of estrogen receptor beta in normal skin, melanocytic nevi and malignant melanomas. J Dermatol 2008; 35: 215-221.
19. Schmidt AN, Nanney LB, Boyd AS, King LE Jr, Ellis DL. Oestrogen receptor-beta expression in melanocytic lesions. Exp Dermatol 2006; 5:971-980.
20. de Giorgi V, Mavilia C, Massi D, et al. Estrogen receptor expression in cutaneous melanoma: a real-time reverse transcriptase-polymerase chain reaction and immunohistochemical study. Arch Dermatol 2009; 145: 30-36.
21. de Giorgi V, Gori A, Grazzini M, et al. Estrogens, estrogen receptors and melanoma. Expert Rev Anticancer Ther 2011; 11: 739-747.
22. Marzagalli M, Montagnani MM, Casati L, Fontana F, Moretti RM, Limonta P. Estrogen Receptor β in Melanoma: From Molecular Insights to Potential Clinical Utility. Front Endocrinol (Lausanne) 2016; 7:140.

23. Beguerie JR, Xingzhong J, Valdez RP. Tamoxifen vs. non-tamoxifen treatment for advanced melanoma: a meta-analysis. Int J Dermatol 2010; 49:1194-1202.

24. Wills S, Ravipati A, Venuturumilli P, et al. Effects of vaginal estrogens on serum estradiol levels in postmenopausal breast cancer survivors and women at risk of breast cancer taking an aromatase inhibitor or a selective estrogen receptor modulator. J Oncol Pract 2012; 8:144-148.

25. Labrie F, Cusan L, Gomez JL et al. Effect of one-week treatment with vaginal estrogen preparations on serum estrogen levels in postmenopausal women. Menopause 2009; 16: 30-6.

26. Harrison RA, Haque AU, Roseman JM, Soong SJ. Socioeconomic characteristics and melanoma incidence. Ann Epidemiol 1998; 8: 327-333.

27. Veierød MB, Weiderpass E, Thörn M, et al. A prospective study of pigmentation, sun exposure, and risk of cutaneous malignant melanoma in women. J Natl Cancer Inst 2003; 95: 1530-1538.

28. Boniol M, Autier P, Boyle P, Gandini S. Cutaneous melanoma attributable to sunbed use: systematic review and meta-analysis. BMJ 2012; 345: e4757.

29. Jensen LB, Hilden J. Sociological and behavioural characteristics of perimenopausal women with an express attitude to hormone substitution therapy. Maturitas 1996; 23: 73-83.

Table 1. Characteristics of the study population by hormone therapy use

	HT non-users *N* (%)	HT users *N* (%)	*P*-value	ET users *N* (%)	EPT users *N* (%)	Tibolone Users *N* (%)	*P*-value
All women	506,389 (74.0)	178,307 (26.0)		80,866 (11.8)	48,117 (7.0)	9,414 (1.4)	
Number of SMM	1,113	363		170	94	28	
Age [a b]	59.0 (50.5 – 67.5)	58.0 (52.5 – 63.5)	<0.001	60.0 (53.5 – 66.5)	56.0 (51.5 – 60.5)	56.0 (52.5 – 59.5)	<0.001
Highest education [b]			<0.001				<0.001
Elementary school	167,959 (33.2)	48,000 (26.9)		23,911 (29.6)	12,713 (26.4)	1,737 (18.5)	
High school	225,632 (44.6)	88,408 (49.6)		39,429 (48.8)	24,498 (50.9)	4,788 (50.9)	
University and higher	92,989 (18.4)	41,034 (23.0)		17,101 (21.1)	10,655 (22.1)	2,846 (30.2)	
Number of children [b]			<0.001				<0.001
0	68,723 (13.6)	15,335 (8.6)		6,569 (8.1)	4,652 (9.7)	810 (8.6)	
1	64,063 (12.7)	22,654 (12.7)		9,549 (11.8)	6510 (13.5)	1,249 (13.3)	
2	179,815 (35.5)	75,571 (42.4)		32,401 (40.1)	20,911 (43.5)	4,410 (46.8)	
3	124,486 (24.6)	45,338 (25.4)		21,466 (26.5)	11,849 (24.6)	2,282 (24.2)	
>3	69,302 (13.7)	19,409 (10.9)		10,881 (13.5)	4,195 (8.7)	663 (7.0)	
Age at first birth [b]			<0.001				<0.001
<20	70,164 (13.9)	28,502 (16.0)		11,415 (14.1)	8,940 (18.6)	1,450 (15.4)	
20-24	203,941 (40.3)	79,361 (44.5)		36,624 (45.3)	21,042 (43.7)	4,180 (44.4)	
25-29	113,643 (22.4)	39,979 (22.4)		19,214 (23.8)	9,695 (20.1)	2,231 (23.7)	
30-34	36,284 (7.2)	11,129 (6.2)		5,196 (6.4)	2,766 (5.7)	542 (5.8)	
>34	13,634 (2.7)	4,001 (2.2)		1,848 (2.3)	1,022 (2.1)	201 (2.1)	
Marital status [b]			<0.001				<0.001
Single	52,945 (10.5)	9,399 (5.3)		3,591 (4.4)	3,307 (6.9)	484 (5.1)	
Married/Partnered	261,626 (51.7)	106,761 (59.9)		48,107 (59.5)	27,748 (57.7)	6,146 (65.3)	
Widow	109,717 (21.7)	29,252 (16.4)		17,348 (21.5)	6,006 (12.5)	933 (9.9)	
Divorced/Separated	82,101 (16.2)	32,895 (18.5)		11,820 (14.6)	11,056 (23.0)	1,851 (19.7)	
Antihypertensives [c]			<0.001				<0.001
Yes	200,270 (39.5)	75,717 (42.5)		38,148 (47.2)	18,425 (38.3)	3,372 (35.8)	
Antidiabetics [c]			<0.001				<0.001
Yes	29,164 (5.8)	7,975 (4.5)		4,505 (5.6)	1,693 (3.5)	276 (2.9)	
Statins [c]			<0.001				<0.001
Yes	119,208 (23.5)	44,214 (24.8)		24,693 (30.5)	9,047 (18.8)	1,887 (20.0)	
Thyroid therapy [c]			<0.001				<0.001
Yes	53,226 (10.5)	25,125 (14.1)		12,069 (14.9)	6,048 (12.6)	1,251 (13.3)	
Region [b]			< 0.001				< 0.001
North	54,743 (10.8)	16,157 (9.1)		7,721 (9.5)	4,002 (8.3)	911 (9.7)	
Mid	43,481 (8.6)	16,529 (9.3)		8,282 (10.2)	4,162 (8.6)	628 (6.7)	
South	401,370 (79.3)	145,620 (81.7)		64,863 (80.2)	39,953 (83.0)	7,875 (83.7)	
ERY [d]			<0.001				<0.001
Low	136,148 (26.9)	47,791 (26.8)		23,808 (29.4)	11,452 (23.8)	2,095 (22.3)	
Medium	233,870 (46.2)	85,535 (48.0)		36,776 (45.5)	23,749 (49.4)	5,047 (53.6)	
High	129,576 (25.6)	44,980 (25.2)		20,282 (25.1)	12,916 (26.8)	2,272 (24.1)	

[a] Median (Interquartile range); [b] Registered at baseline; [c] Prescribed anytime during follow-up; [d] Sun exposure measured as erythemally weighted UV radiation (ERY). HT: Hormone therapy, ET: Estrogen therapy, EPT: Estrogen-progestin therapy, SMM: Skin malignant melanoma, ERY: erythemally weighted UV radiation. Some numbers do not add up to total number due to missing values.

Table 2. Use of hormone therapy and risk of skin malignant melanoma.

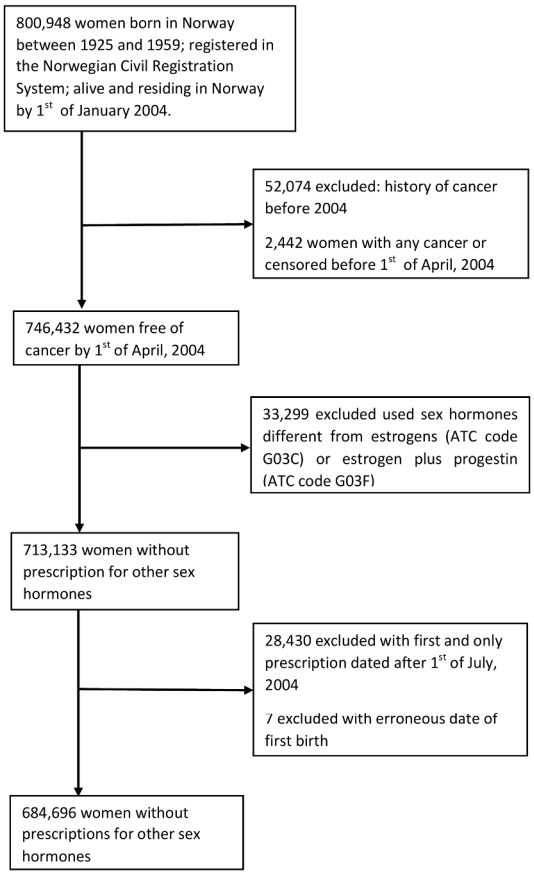
		All women			Women ≥ 55		
		PY	cases	RR (95% CI)	PY	cases	RR (95% CI)
Status	Non-use	2,451,037	1,113	Reference	1,552,601	788	Reference
	Current use	450,912	248	1.19 (1.03 – 1.37)	299,326	188	1.23 (1.05 – 1.45)
	Past use	240,929	115	1.00 (0.82 – 1.21)	171,716	90	1.00 (0.80 – 1.25)
	Ever use	691,840	363	1.12 (0.99 – 1.26)	471,041	278	1.15 (1.00 – 1.32)
HT-type[b]	Non-use	2,451,037	1,113	Reference	1,552,601	788	Reference
	Estradiol	152,488	107	1.48 (1.21 – 1.81)	109,375	82	1.45 (1.15 – 1.82)
	Estriol	38,309	28	1.33 (0.91 – 1.95)	36,474	28	1.36 (0.93 – 1.99)
	ET[a]	190,797	135	1.45 (1.21 – 1.73)	145,850	110	1.43 (1.17 – 1.74)
	Tibolone	29,705	19	1.39 (0.88 – 2.18)	18,898	18	1.88 (1.17 – 3.01)
	EPT	140,204	57	0.91 (0.70 – 1.19)	87,350	36	0.84 (0.60 – 1.17)
	Mixed users[c]	90,205	37	0.94 (0.68 – 1.31)	47,228	24	1.03 (0.68 – 1.55)
Type of combined regimen[b]	Non-use	2,451,037	1,113	Reference	1,552,601	788	Reference
	Continuous EPT	119,981	44	0.80 (0.59 – 1.09)	83,068	33	0.80 (0.56 – 1.14)
	Sequential EPT	20,223	13	1.70 (0.98 – 2.94)	4,281	3	1.53 (0.49 – 4.76)
Route[b]	Non-use	2,451,037	1,113	Reference	1,552,601	788	Reference
	ET[a] oral	68,848	49	1.45 (1.09 – 1.93)	54,038	41	1.42 (1.03 – 1.94)
	ET[a] vaginal	103,050	74	1.44 (1.14 – 1.84)	80,644	62	1.45 (1.12 – 1.88)
	Estradiol transdermal	11,267	5	1.01 (0.42 – 2.42)	5,987	1	0.33 (0.05 – 2.38)
	EPT oral	137,068	55	0.90 (0.69 – 1.18)	85,908	35	0.83 (0.59 – 1.16)
	EPT transdermal	2,232	2	2.00 (0.50 – 8.00)	1,090	1	1.79 (0.25 – 12.70)
	Mixed users[c]	128, 447	63	1.11 (0.86 – 1.43)	71,658	48	1.34 (1.00 – 1.81)

All RRs were adjusted for age, number of children, age at first birth, education, marital status, sun exposure, use of antihypertensives, antidiabetics, statins, thyroid therapy. HT: Hormone therapy, PY: person-years, RR: incidence rate ratio, CI: confidence interval, ET: estrogen therapy, EPT: estrogen-progestin therapy. [a]Estradiol and estriol combined. [b]Based on current use.

Table 3. Current ET and EPT use and risk of melanoma according to some population characteristics

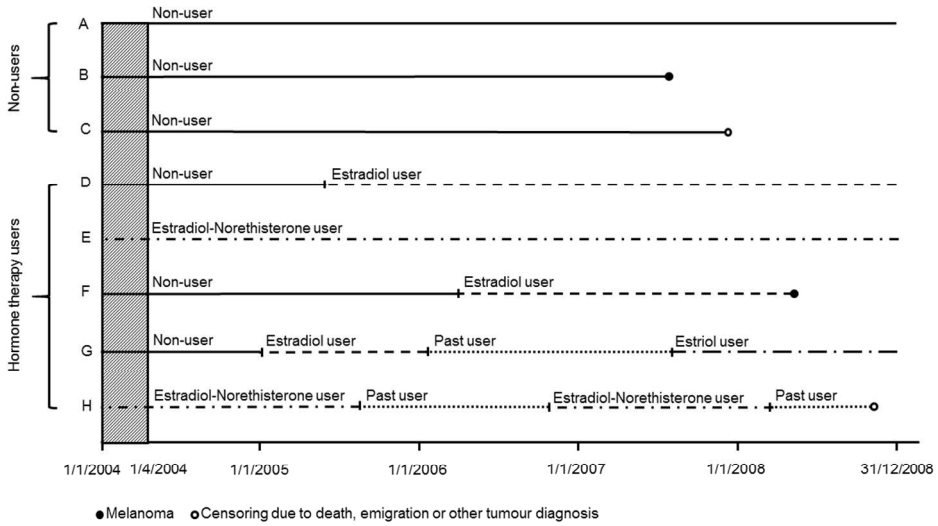
	ET RR (95% CI)	EPT RR (95% CI)
Age (years) [a]		
45-53	1.83 (1.18 – 2.83)	1.30 (0.82 – 2.08)
54-62	1.62 (1.20 – 2.18)	0.89 (0.60 – 1.33)
63-79	1.34 (1.02 – 1.74)	0.68 (0.37 – 1.24)
Sun exposure [b]		
Low	1.03 (0.64 – 1.66)	1.12 (0.62 – 2.01)
Medium	1.67 (1.31 – 2.13)	1.01 (0.70 – 1.44)
High	1.35 (0.97 – 1.88)	0.67 (0.39 – 2.84)
Age at first birth (years) [a]		
	1.46 (0.83 – 2.60)	0.79 (0.32 – 1.94)
No child	1.67 (1.17 – 2.39)	1.04 (0.61 – 1.79)
< 21	1.44 (1.05 – 1.97)	1.07 (0.68 – 1.66)
22-25	1.32 (0.96 – 1.82)	0.61 (0.33 – 1.11)
> 25		
Education		
Elementary school	1.40 (0.97 – 2.02)	1.07 (0.62 – 1.83)
High school	1.43 (1.11 – 1.84)	0.86 (0.59 – 1.25)
University and higher	1.54 (1.07 – 2.23)	0.90 (0.52 – 1.55)

ET and EPT estimates were mutually adjusted, in addition to adjustment for parity, marital status, use of antihypertensives, antidiabetics, statins and thyroid therapy. ET: estrogen therapy, EPT: estrogen-progestin therapy, RR: incidence rate ratio, CI: confidence interval. [a]Age was divided in tertiles. [b]Residence county-level sun exposure, categorized as low (230-300 kiloJoule per square meter (kJ/m2)), medium (320-360 kJ/m2), high (370-400 kJ/m2).



Flow chart of study participants

215x279mm (300 x 300 DPI)



Follow-up definition of study participants

338x190mm (96 x 96 DPI)