Alma Mater Studiorum – Università di Bologna

DOTTORATO DI RICERCA IN

Scienze chirurgiche

Ciclo XXXIII

Settore Concorsuale: 06/D4

Settore Scientifico Disciplinare:MED/35

Female melanoma: the role of ovarian stimulation and antioestrogens cancer therapy

A retrospective study with immunohistochemical oestrogen receptors alpha and beta analysis.

Presentata da: Federica Scarfi

Coordinatore Dottorato **Supervisore**

Prof.ssa Annalisa Patrizi **Prof.ssa Emi Dika**

Esame finale anno 2021

Tables of contents

Thesis outline

This thesis is organised as follows: Chapter 1 outlines the research background, objectives and research scope. A comprehensive review of the literature regarding the melanoma sex disparities and the role of oestrogens and oestrogen receptors on female melanoma is discussed in Chapter 2. Details of the methods are discussed in Chapter 3, such as the statistical analysis of the population study and the characterisation techniques for oestrogen receptors immunohistochemical staining. Chapter 4 highlights the results of our research. In Chapter 5, a discussion on the findings of the research is carried out. Finally, Chapter 6 concludes the thesis and provide some recommendations for future prospectives.

Chapter 1

1.0 Research background:

Cutaneous melanoma (CM) is the most lethal type of cutaneous cancer[1]. In the last two decades, the incidence of CM has significantly risen in fair-skinned populations[2]. In 2019, it was estimated that 5.699 new CM cases occurred among females in Italy. CM represents the third most common cancer in Italian women under 49 years old[3]. The hypothesis that sex hormones can have a role in melanoma genesis was postulated based on two main epidemiologic observations: the difference in onset age of melanoma among genders and the better survival of women. In the last decades, many molecular studies performed on animal models and cell lines confirmed that sex hormones have a part in melanogenesis and melanoma genesis[4]. However, these findings seem not to correlate with epidemiological data directly, and many controversies are present regarding the role of exogenous oestrogens intake and endogenous hormonal status in female melanoma[5,6].

In recent years, oncology research focused its attention on the identification of oestrogen receptors in various tissues, some of them believed not to be responsive to sex hormones, but the site of indirect estrogenic action[7]. In some of these, such as the prostate and brain, the predominant oestrogen receptor is ERβ (Estrogen Receptor beta). On the contrary, its reduced expression has been found in the tumorigenesis of other organs, including breast, colon and ovary[8-10]. ERβ has an antiproliferative, proapoptotic action and is also involved in the regulation of the expression of numerous molecules of adhesion.[11,12] As well-known, the skin is also an oestrogen-responsive tissue. Some descriptive studies documented the presence of ERβ in the skin and as a predominant receptor in melanocytes both in dysplastic naevi and melanomas.[13-15] Moreover, in melanoma ERβ, seems to be inversely correlated with Breslow thickness in melanoma: in particular, thick melanomas (> 1 mm) display a lower expression of the receptor than thin ones (≤ 1 mm)[16,17]. This inverse correlation suggests that the loss of ERβ may influence the aggressiveness of melanoma and its progression, thus identifying a potential prognostic marker. However, the real significance of this correlation is still under investigation.

On the other hand, ERα was not detected with immunohistochemistry in benign or malignant melanocytic lesions even if its presence was revealed with mRNA reverse-transcriptase polymerase chain reaction[17]. For this reason, ERα presence in primary CM is still an unresolved issue.

In a preliminary analysis, we investigate, through immunohistochemistry, the presence of ERα and progesterone receptor (PR) in 28 female patients. Of these, 14 patients had received a diagnosis of melanoma (7 patients) or melanocytic nevus with severe atypia (7 patients) in pregnancy or the six months after childbirth or following repeated IVF courses in the previous year. The remaining 14 patients, of the same age and ethnicity, had removed a melanoma (7 patients) or a melanocytic nevus with severe histological atypia (7 patients). This latter did not have any history of hormonal stimulation or oral oestrogen-progestogen intake. Upon immunohistochemical analysis, we found that only two women showed a significant cytoplasmatic positivity for ERα. Upon immunohistochemical investigation, the CMs that were strongly positive were of the nodular type and with Breslow thickness> 1 mm: 1.2 mm and 1.4 mm, respectively. The remaining cases did not show positivity for ERα and PR[18]. These preliminary results may suggest the existence of a correlation in women between cytoplasmatic ERα and the onset of melanoma with a histopathological feature of aggressiveness, the nodular histotype.

1.1 Objectives

The primary objective of our study is to investigate the clinical and histopathological features of melanoma in this female population, focusing on two main subgroups: women in fertile age and women in menopausal age. We evaluated all female patients with histological diagnosis of cutaneous melanoma diagnosed from 01/2008 to 12/2018 at the Sant'Orsola-Malpighi Hospital and evaluated at least once at the Melanoma Unit of the Dermatology Department, University of Bologna.

The secondary objective is to evaluate the expression of ERα and ERβ by immunohistochemical analysis (anti-ERα and anti-ERβ antibodies) in this cohort. The investigation involves women who underwent to hormonal stimulations for medically assisted procreation (MAP), women in antioestrogens cancer therapy for breast cancer and two control groups matched for age and stage.

The tertiary objective is to evaluate the expression of ERβ and ERα for each group in correlation with the Breslow thickness and other relevant histopathological (histotype, ulceration, regression, mitotic index), clinical (age, skin photodamaging, number of nevi, melanoma location) and dermoscopic characteristics (atypical network, blue-white veil, atypical vascular pattern, irregular streaks, irregular dots/globules, irregular blotches and regression structures).

1.2 Research scope

In this study, we planned to obtain data regarding the ERs presence in melanoma cells of women underwent to hormonal stimulations for medically assisted procreation (MAP) and women in antioestrogens cancer therapy. We look for any possible correlation between clinical and histopathological characteristics and these two different hormonal scenarios among our study population. These data will validate our preliminary results regarding the cytoplasmatic presence of ERα in the nodular melanoma of the patients with a history of ovarian stimulations. We also plan to verify if the expression of ERβ by immunohistochemical analysis (anti-ERβ antibodies) correlate inversely with the Breslow thickness or with other histopathological prognostic characteristics (histotype, ulceration, regression, mitotic index). Our main aim is to try to shed some lights on the oestrogen receptors presence and their role in female melanoma hormonal status.

2.0 Women melanoma and melanoma sex disparities

Melanoma has a different behaviour among gender regarding the age of incidence, body site affected and survival[19,20]. In particular, women are more like to develop melanoma in the premenopausal age, while the incidence in the postmenopausal period is significantly lower. It is well known that females more commonly have melanoma on the lower extremities than men who often display it in the trunk, and this is true regardless of CM invasiveness[21]. Moreover, women have a better prognosis with a survival advantage over men[22]; this makes the female gender an almost protective factor against death from melanoma[23]. Indeed, this benefit in survival seems to be not linked the known prognostic factors such as Breslow thickness, mitoses, age at diagnosis, morphology, body part affected, ulceration, lymph node analysis as demonstrated in observational studies and clinical trials[24-26]. For these reasons, the female gender is considered worldwide an independent favourable prognostic factor for melanoma survival[27].

2.1 Behavioural factors

Many behavioural differences among gender were investigated to assess their role in these melanoma sex disparities. Differences towards sun exposure and health care were the main investigated factors. Women are generally more prone to sunbathing[28]. On the other hand, men are more likely to adopt riskier behaviour concerning preventive care and medical compliance[29].
Even nutritional habits may have a role as shown in a recent work by Lukic M. et al., where the authors found that a moderate intake of filtered coffee reduces the risk of CM among a large cohort of Norwegian women[30].

Regarding the impact of ultraviolet (UV) exposure, a vast population study finds that UV is a significant causative factor for melanoma but only for women older than 44 years. In this study, the UV factor was not able to fully explain the incidence of melanoma in younger women[31]. Moreover, the latest findings suggest a relationship between UV index and male gender, not with female one[32].

2.2 Genetic factors

Numerous recent studies indicated that these gender melanoma differences are more likely due to biological rather than the overcited behavioural factors, also considering that these disparities are observed worldwide, regardless of sun exposure levels.

Biological mechanisms proposed in melanoma sex differences are mainly linked to immune system actions, oxidative stress reaction ability, vitamin D metabolism, genetic sex chromosome expression and hormonal regulation[33].

Regarding genetics, it is meaningful to highlight the role of the selective inactivation of the X chromosome in female body cells that results in somatic mosaicism with a higher genetic diversification. X chromosome alone has many oncogenes and tumour suppressor genes that are involved in cancerogenesis[33,34].

Indeed, from childhood, there are sex differences in mole distribution, under genetic control in women, that reflect the distinctive melanoma distribution among sexes in adulthood[19,35]. Epigenetic effects linked to X inactivation are likely to influence the nevi distribution[36]. Moreover, in patients affected by Turner syndrome (XO) is expected the presence of a large number of moles on limbs and a higher risk for melanoma development [19,37].

Furthermore, in the Spanish population, a sex-specific genetic effect was observed to be associated with sunlight sensitivity, pigmentation and melanoma risk[38].

Finally, the mutation burden of CM is significantly higher in male compare to female, mirroring a female immune system better capacity of effective antitumor surveillance[39].

2.3 Immune factors

Melanoma is a high immunogenic cancer, as also demonstrated by the higher risk for CM in patients with systemic immunosuppression included transplanted ones[40]. For this reason, an important topic of sex disparities toward melanoma is related to the aged and sex-hormone changes in immune system[41].

Especially after puberty in both adaptative and innate immunity, there are changes between males and females. These impact the incidence and pathogenesis of cancer as well as the efficacy of cancer immunotherapy. Ageing in the innate immune system, with its consequences, seems to be faster in men than in women [42,43]. Generally, Ig G and Ig M levels, CD4+ T cells, CD4/CD8 ratio and slightly CD 3+ cells T-lymphocytes are found higher in females; on the contrary, a lower Th1 response and IL-2 production characterised the males immune response[44-45].

Sex hormones play a crucial role in the modulation and homeostasis of the immune system, considering that the ERs are expressed by T cytotoxic cells, B cells, dendritic cells and others. The exact mechanisms through which this interaction occurs are complex, as it also involves receptors other than ERs and it is still under investigation[46].

In mice model oestrogens enhances the production of molecules, through specific gene transcriptions, that are implicated in the prolonged survival and activation of B cells[47].

Oestradiol is the primary steroid molecule involved in the relationship between oestrogens and the human immune system. It is proved that oestradiol increases Ig G and Ig M levels in peripherical monocytes [33,48] and its concentration represent a regulator in the adaptative immune function as a low concentration elicits a Th1 response. Meanwhile, a high level evokes the humoral immunity and a Th2 response[49].

During the last decades with the beginning of the immunotherapy era in melanoma treatment, it has been found a relationship between sex hormones and the PD-1 signalling pathway. In particular, oestrogens can modulate the programmed cell death ligand 1 (PD-L1) and Treg-linked B7-H1 suppression, providing a better response through anticancer therapy with anti-PD-1 agents[49,50].

2.4 Hormonal factors

A large body of the literature maintains the positive function of oestrogen against melanoma progression. Sex hormones, particularly oestrogens, have been investigated as the main mechanism involved in the gender disparities, considering the difference in the cellular hormonal backgrounds. The exposure to oestrogens is physiologically part of the woman life, and it depends on reproductive factors: mainly menarche, parity and menopause. A relationship between endogenous hormones and melanoma is still debated.

Besides, the data from the published studies are often affected by the study design. The majority of them are retrospective cohort study that can have recall and selection bias.

The first works seem to prove a possible influence of female reproductive factors on melanoma[51], although in the last few years, larger scientific studies do not show any relationship [5,52,53]. Indeed, recently Olsen CM et al.[54] have found no association between melanoma incidence and age at menarche, parity, menopausal status at baseline and age at menopause in their prospective cohort study on Australian women. A similar result was displayed by Støer NC et al.[55] within their large (172.478 women) nationwide population-based cohort in Norway.

Donley GM et al.[56] instead, demonstrated an increased melanoma risk associated with early age at menarche and late age at menopause, hypothesising that endogenous oestrogen exposure during childhood can increase during life photocarcinogenicity.

About parity, the literature is even more controversial: parity at a younger age is generally considered a protective factor as well as higher parity even if a bias linked to socio-economic confounders and individual lifestyle can be present[5,52,57-60].

Regarding benign gynaecological diseases and melanoma risk, there is no sufficient evidence considering the no-large number of epidemiological studies that investigated this topic [61]. A prospective epidemiologic study found a statistically significant increase of melanoma risk among women with endometriosis (relative risk 1.62) and fibroma (relative risk 1.33)[62]. Recently these data were confirmed by the same study group finding also a higher risk for the non-melanoma skin cancers[63]

The effect of pregnancy in melanoma is still an unresolved issue, especially concerning the disease prognosis during motherhood[64].

It is known that pregnancy is accompanied by numerous physiological changes, including skin hyperpigmentation in almost 85% to 90% women[65,66]. In some early reports, changes in the moles have been observed during gestation. In particular, the most frequently observed modification concerned the colour of the nevi, which turned towards darker shades with pregnancy, and their size. These variations were in many cases, self-perceived and were reported in about 30% of women[67]. Is recognised that there is no evidence of physiologic changes in nevi during pregnancy. The most visible changes are found in skin lesions located in the breast and abdomen, and they are due to their growth with normal skin expansion[68]. Only one histopathological study found an increase in dermal mitoses and Ki-67 proliferation index among dermal nevi excised during pregnacy[69]. There are prove of dermatoscopic changes, but they are transient, and none are suggestive of melanoma[70]. In pregnant patients with a history of melanoma and multiple dysplastic nevi, dermatology examinations should be close also in the early post-partum[71].

The scientific literature encompasses many epidemiological studies that looked at the relationship between pregnancy and CM. There are many limitations in data interpretation linked to the type of statistical analysis and study design, the number of participants, the definition of the so-called “pregnancy-associated melanoma” (PAM). Moreover, in case-control studies, age-matched control groups do not take into account confounding factors such as ulceration, melanoma staging, anatomic site of lesions, phototype and sun exposure[72].

Concerning the CM diagnosis during pregnancy, a large body of literature do not reveal a poorer prognosis in pregnant patients[73-76].

A large Swedish registry-based study compared mortality between PAM (defined as a period ranging from pregnancy to two years after child delivery) and women not diagnosed near childbirth: the cause-specific mortality did not differ between the two groups adjusting for age, period, education, parity, and tumour location (adjusted hazard ratio 1.09, 95% confidence interval 0.83-1.42) [77].

In a previous review by Lens M and Bataille V[5] of 10 case-control studies emerged that pregnancy does not impact melanoma survival and also not increase the risk of a second primary CM. Moreover, the authors

concluded that there is no reason for physicians to suggest a delay of a subsequent pregnancy in stage I melanoma patients. This advice is accepted in almost all European countries[78].

On the contrary, a recent single-institution study was PAM is defined as melanoma diagnosis during gestation or within one year after childbearing, maintains pregnancy is a negative factor with a five-, seven-, and ninefold increase in mortality, metastasis, and recurrence, respectively, when compared with controls [79].

Conversely, in a large body of the literature, there are no findings that CM prognosis is changed when women melanoma is diagnosed up to five years following childbirth[77,80,81], except for one study that shows an increased risk of death in the first year postpartum[82]. A delay in diagnosis may cause this finding.

In women life, exogenous oestrogens sources are mainly represented by oral contraceptives (OCPs), hormone replacement therapy (HRT) and less frequently by ovarian stimulation for MAP.

Starting from the observation that acquired skin hyperpigmentation can also be present in women under OCPs[83] and that keratinocytes hold ERs[84], multiple studies have assessed the risk of melanoma associated with OCPs. Some works display an elevated risk, especially with prolonged use[85]. These data can be partially explained by the behaviour through sun exposure and by the photosensitizing property of these drugs[86]. Furthermore, a current work finds a positive association between the use of OC and HRT and one of the most important phenotypic risk factors of CM: a high melanocytic nevi count[87].

The two main meta-analyses of epidemiologic observational studies suggested no evidence for a higher melanoma risk with the use of OCPs[52,88]. A pooled analysis of ten case-control studies by Karagas, M.R. et al.[89] displayed the same result: no relation between melanoma incidence and duration of oral contraceptive use was found even considering other confounder factors such as hair colour, sun sensitivity, family history of melanoma and sun exposure. The post-hoc analysis performed by the Women's Health Initiative trials on HRT in postmenopausal women shows no increased risk for melanoma and non-melanoma skin cancer[90]. Meanwhile, the large EPIC (European Prospective Investigation into Cancer and Nutrition) prospective cohort study found a weak, statistically nonsignificant association between OCPs or HRT use and melanoma risk[91]. Besides, a recent case-control study by De Giorgi V et al[92] shows that OCPs or HRT have even a protective effect against CM. A clear relationship between the duration of oral contraceptives intake and the age of onset and the incidence of melanoma is still debating.

The association between ovarian stimulation and CM risk is another controversial issue. Actually, its importance is increasing along with the increment of assisted reproductive techniques (ART) use. Worldwide almost 1% of birth are due to ART utilize[93]. The cancer risk with other hormonal treatments is not comparable because of the high or very high (over ten-fold comparing to normal ovarian cycle[94]) oestradiol levels, progesterone supplementation and antioestrogen (clomiphene) or gonadotrophins intake[95]. A recent work [96] analysing a large longitudinal cohort of US women (113,226 women, including 53,859 women without prior ART treatment), showed non-significant higher risks for CM after nearly five years of follow-up compared to the general population, considering also confounding effect of infertility. Most of the published studies had a

small number of cases, and consequently, they are not able to reach statistical significance. Two larger studies with respectively 42 and 112 participants displayed an almost 5% increased risk of CM among women using fertility drugs[97,98]. A current review[99], examining potential associations between in vitro fertilization (IVF) and CM, do not reveal a consistent relationship between IVF and CM.

Nevertheless, the authors highlighted as the data indicates a potential increased risk for MM in ever-parous women undergone IVF. Some studies demonstrated an increased CM in women undergoing ovarian stimulation related to clomiphene citrate, a drug commonly used in controlled ovarian hyperstimulation[100,101]. Meanwhile, the majority of works on fertility drugs and cancer showed no association with CM[102-104].

Lastly, a possible scenario is characterized by antioestrogen cancer therapy intake. Indeed, epidemiological studies maintain that women with previous breast cancer (BC) have a higher risk of CM, and this risk is greater for patients who do not receive anti-estrogen therapy[105,106]. Aromatase inhibitors (AIs) are both therapeutic and adjuvant agents in BC. Early studies found that the aromatase enzyme is expressed in melanoma, but no relationships were demonstrated between the presence of the enzyme and prognosis[107,108].

2.5 Estrogen receptors and melanoma

Estrogens are known to contribute to skin processes such as wound healing, thickening epidermis, mediating inflammatory disorders and preventing from photoaging [109]. Skin cells are targets of these hormones, and they are known to express ERs that are responsible for cutaneous estrogenic effects through their binding and the consequent activation of the two specific receptors, called estrogen receptors alpha (ERα) and estrogen receptor beta (ERβ), which have similar structural and functional characteristics. ERs belong to the nuclear steroid hormone receptor superfamily and exert their role as ligand-activated transcription factor upon homo-heterodimers. Structurally, ERα and ERβ are respectively 595 and, 530 amino acids and their weight are respectively 67kDa and 59kDa. They are both characterized by DNA binding and a ligand-binding domains[110](Figure 1).

In particular, the molecular structure is composed of:

- an N-terminal domain (A / B domain), highly variable both in sequence and in length, containing the AF1 region (Activation Function-1) a regulatory sequence[111]; it includes a zinc-finger structure that is responsible for binding target sequences.

- a highly conserved central domain (DNA binding domain, DBD or C); it is crucial for chromatin binding.

- a hinge domain (D domain); important for translocation in the RE nucleus, it is unmasked upon oestrogens binding and, it connects the C and E domains. Moreover, it can bind the chaperone proteins.

- an E domain, which binds the hormone (ligand-binding domain, LBD).

- an F domain at the C-terminal region. It contains the AF2 region (Activation Function-2) another regulatory sequence. Together with the E domain, it also has binding sites for coactivators and co-inhibitors.

AF1 can be active without the presence of the steroid hormones. The main architectural difference between the two receptors is that ERβ has a shorter amino-terminal domain than ERα. The ERs are encoded by two different genes that are located on chromosome 6 for ERα and chromosome 14 for ERβ.
Regarding ERα, besides the full-length isoforms, there are several shorten isoforms that are the results of alternative splicing or different start codons. There are unable by themselves to control genes transcription, but they can arrange heterodimers with the full-length form and inhibit its action. Meanwhile, the shorter isoform (ER-α36) is responsible for membrane-initiated signaling[112].
On the other hand, ERβ has five isoforms that differ from the full-length protein for the composition of the C-terminal region. ERβ isoforms can abolish ERα function by forming a heterodimer with it[113].
All the ERs are mainly located in the nucleus, though some can be associated with the cell surface membrane. These latter are rapidly activated by estrogenic exposure. ERα and ERβ are activated with different affinity, by various both natural and synthetic ligands.
The binding between intracellular ERs and their ligands determines *the genomic pathway* with nuclear translocation and the direct interaction with chromatin at specific target sequences, known as oestrogen response elements (EREs)[114]. Recent reports assess that in 35% of genes, involved in estrogenic biological effect, there is a lack in ERE-like sequences[113,115]. In these the mechanism leading to genetic expression is called "transcriptional cross-talk", and it consists on an interaction between oestrogen receptor complexes and transcription factors such as the stimulating protein-1 (Sp-1) and the activator protein-1 (AP-1)[116,117].
In *the non-genomic pathway*, instead, the binding is on the cellular membrane, and it triggers a cascade of intracellular signalling events leading to gene expression with or without a direct link between estrogen-receptor complexes and DNA. The changes in the cytosolic signalling, leading to the activation of four different protein-kinase cascades, including the RAS/BRAF/MEK axis[118,119]. Strikingly, the MAPK pathway contains the molecules that are targeted by the BRAF inhibitors, such as vemurafenib, and the MEK inhibitors, such as dabrafenib, that are the first-line treatment for V600E mutant BRAF-harboring melanomas. To note, BRAF is known as the main protein driver in melanoma growth, occurring in about 50% of CM, especially in young patients with tumour located mainly on sun-exposed areas[108]. The biological importance of the interaction between MAPK activation and ERα molecular mechanism is also demonstrated in vivo in animal models. Moreover, a large body of literature maintains strong communication between steroid hormones and growth factor receptors, having a great impact on cellular response to physiological or pathological situations[108,120].
The most involved estrogen receptor in *the non-genomic pathway* is the G protein-coupled estrogen receptor (GPER1), identified by molecular cloning methods and isolated almost two decades ago. GPER1 is a G protein-coupled membrane receptor that acts independently from ERs. Their agonists are G1 and 17β-estradiol[121]. It is crucial for many cellular processes, and it is also associated with cancers genesis and growing. Most important, in cutaneous tissue it is involved in melanogenesis regulation, and it is expressed in melanoma

cells[108, 122,123]. In vitro GPER1 agonists are able to inhibit melanoma cell proliferation[124]. Furthermore, when GPER1 is co-expressed with ERβ in melanoma, the histopathological parameters are more favourable (lower Breslow thickness, lower mitotic rate and higher presence of peritumoral lymphocyte infiltration)[125].

There is growing evidence that alters function in estrogen receptors signalling may have a role in cancer genesis, development and evolution. In the same way, it is well known that ERα plays a part in tumorigenesis by increase cellular growth while, on the contrary, ERβ has an antiproliferative action. ERα is the most representative ER in the human epidermis, and typically its presence is reduced in both benign and dysplastic nevi, but also in metastatic or primary melanoma, as well as in pregnancy-associated melanoma. It is usually rarely detectable with traditional immunohistochemistry analysis. Its amount in CM cells seems to not match with the pathophysiology of melanoma precursor lesions or melanomas themselves[126]. In contrast, our preliminary immunohistochemical study showed a strong cytoplasmic localization of ERα in melanoma of women who experienced ARTs[18].

Furthermore, the melanoma susceptibility, as well as the response to therapy, can be partially influenced by the presence of ERα single-nucleotide polymorphisms[127]. At the same time, ERβ is the predominant ER subtype in all melanocytic lesions: moles, dysplastic naevi and melanoma[128]. In addition, ERβ tends to diminish in vitro after UV exposure and is more present within thin melanocytic lesions such as naevi with severe dysplasia and in situ melanoma. ERβ has been suggested as a CM marker[129] due to its exact inverse correlation with the most powerful CM prognostic factor: the Breslow thickness[130]. Data regarding the potential protective function of ERβ has also been found in breast, ovarian and prostate tumours as well as in colon cancer[131-140].

An immunohistochemical study indicates that ERβ is generally more present in women CM, especially pregnant ones, in comparison to men. The work of Schimdt et al. maintain these findings evaluating ERβ expression in three categories: pregnant women, non-pregnant women, and men of patients stage and age-matched[13].

3.0 Materials and methods

3.1 Study design

A cross-sectional study is performed at Dermatology section, Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Italy. Written informed consent was obtained for all study participants. The Ethics Committee approved the study of the Sant'Orsola-Malpighi Hospital and University of Bologna (protocol number 309/2019Sper.AOUBo).

3.2. Study population

We performed a retrospective analysis of patients with a histologically confirmed diagnosis of invasive or in situ CM received from 01/2008 to 12/2018 at the Sant'Orsola-Malpighi Hospital and evaluated at least once

at the Melanoma Unit of the Dermatology Department, University of Bologna. No restraints were applied to Breslow thickness.

The inclusion criteria were:

- Female gender
- Age >18 years

The exclusion criteria were:

- Patients with unknown primary CM
- Breslow thickness not available

For patients with multiple primary melanomas, the most invasive tumour was included in the analysis. Patient information was recorded through medical data. In particular, the following epidemiological data were evaluated in the study: age at first CM diagnosis, family history of melanoma, Fitzpatrick skin type, date of CM diagnosis, CM body site, previous excision of non-melanoma skin cancer, breast cancer personal history, age at menarche, hormone therapy (both OCPs and HRT) duration, number of pregnancies, age at first pregnancy, number of abortions, use of ARTs, age at menopause. Medical data taken from the patient records were as follows: Breslow thickness, number of mitoses, ulceration, histotype, type of growth, presence of satellite nodules, tumour-infiltrating Lymphocytes (TIL), regression, lymphovascular invasion, presence of metastasis. To investigate the clinical and histopathological characteristics of female melanoma in different women hormonal status, we divided our population study into two main subgroups: women in fertile age versus women within menopausal age.

The secondary objective was to evaluate the expression of ERα and ERβ by immunohistochemical analysis (anti-ERα and anti-ERβ antibodies) among selected patients of our study population.

3.3 Statistical analysis

All the statistical analyses were performed using R version 4.0.2 (https://www.r-project.org/). Individual and tumour characteristics were summarized through descriptive statistics, i.e. mean and standard deviation (SD) for quantitative variables, and absolute and percentage frequencies for categorical variables.

Tumour characteristics (all categorical) were compared between two different age groups (Age ≤ 52 years and Age > 52 years) and between individuals with or without a medical history of breast cancer, by means of a Fisher exact test. A statistically significant association was defined as a p-value of less than 0.05.

Statistically significant associations were also visually displayed in the respective tables.

3.4 Patients selection for immunohistochemical analysis

The selection of patients applicable for immunohistochemical analysis of paraffin-embedded archival tissues was based on:

- Positive anamnesis for hormonal stimulations for medically assisted procreation (MAP) or antioestrogens breast cancer therapy intake
- Paraffin-embedded tissue-blocks available at the Dermatology Unit of the Policlinic Sant'Orsola-Malpighi
- Negative family history of melanoma
- No family cancer syndromes
- No BCRA1 or BCRA2 mutation detected upon breast cancer gene mutation screening

The investigation also involves other two control groups of women respectively on pre and post-menopausal status of the same age and melanoma staging.

3.5 Tissue samples and histological evaluation

The melanoma material included in the study were obtained by surgical excision carried out at the Skin Tumour Center and Melanoma Unit of the Dermatology Unit of the S. Orsola - Malpighi Polyclinic of Bologna.

The tissue, consisting of FFPE blocks, used for the study was that not dedicated to the routine diagnostic process and not reserved for molecular analysis in case of disease progression.

For histological evaluation, tissue samples had been fixed in 10% formalin, embedded in paraffin at pH 7.4 and stained with Haematoxylin and Eosin (H&E).

All the selected cases were analysed and re-staged by an expert pathologist according to 2018 WHO Classification of Skin Cancer and the 8th edition of the AJCC Cancer Staging Manual and the 2020 CAP melanoma reporting protocol [141-143]. The following clinical-pathological features of primary melanoma have been evaluated:

- Melanoma subtype
- Ulceration
- Growth phase
- Breslow thickness (mm)
- Mitotic count
- Tumour infiltrating lymphocytes-TILs
- Regression (<or >75%)

- Angioinvasion
- Perineural infiltration
- Folliculotropism
- Microsatellites
- Margin status
- pT staging

Two slides three µm thicker were obtained from each block and subsequently used for immunocytochemical receptor research.

Once the two slides were obtained for each patient, one for the detection of ERα and one for ERβ estrogen receptors, the material was transferred to the Unit of Anatomy and Pathological Histology of the S. Orsola - Malpighi Polyclinic where the immunohistochemical analysis was carried out.

3.6 Immunohistochemical staining and molecular analysis

The immunohistochemical analysis was conducted with the support of the VENTANA BenchMark ULTRA automated slide stainer (Ventana Benchmark Ultra System, Diagnostics Roche, Hoffmann-La Roche; Basel, Swiss) with the Opti View DAB IHC Detection Kit (Ventana).

Detection Kit is an indirect, biotin-free system for detecting specific primary mouse and rabbit antibodies bound to an antigen in paraffin-embedded tissue sections that are stained on the VENTANA automated slide stainers and visualized by light microscopy. It also uses a cocktail of enzyme-labelled secondary antibodies, which localize the bound of the primary antibody. The cocktail is recognized by an enzyme-bound tertiary antibody that is visualized with hydrogen peroxide substrate and 3, 3' – diaminobenzidine tetrahydrochloride (DAB) chromogen, which produces a brown precipitate detectable by light microscopy.

The staining protocol consists of several steps during which the reagents are incubated for predetermined periods of time and at specific temperatures. At the end of each incubation phase, the Ventana BenchMark Series instrument washes the sections in order to remove the unbound material and applies a liquid coverslip solution that minimizes the evaporation of the reagents acquired from the slide. The results are interpreted with an optical microscope and are useful for the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

In particular, the slides were deparaffinized in Bio-Clear (Bio-Optica, Milan, Italy) and hydrated with decreasing ethanol concentrations and distilled water. The immunostaining protocol included the antigen retrieval by multi-steps pre-treatment with CC1 solution (Ventana), the block of the endogenous peroxidases with 2.0% H202 in distilled water (5 minutes) and the incubation with the antibody for ERα (clone SP1,

Rabbit Monoclonal, Ventana, dilution ready to use-RTU, Ventana Medical Systems, Tucson, Arizona) and ERβ (Clone ERb455, Mouse monoclonal, dilution ready to use-RTU, Ventana Medical Systems, Tucson, Arizona) respectively for 32' and 40' at 37° followed by DAB staining (Opti View DAB IHC Detection Kit, Ventana).

The precipitated chromogen indicates the presence of the antigen and the intensity of the reaction is proportional to the amount of antigen present.

If the antigens are present in the cytoplasm of the cells, the staining can involve the entire cytoplasm or only a part of it, depending on the antigenic content.

In our investigation, Nuclear and cytoplasmatic staining was separately evaluated and scored by the percentage of stained tumour cells; the staining was further classified as 0 (≤20%), 1 (21%-50%), or 2 (≥50%), as previously reported[13,15,18].

The most challenging aspect of immunohistochemistry is slide evaluation. In the interpretation of the results, both the specific antigen staining and the non-specific background staining must be evaluated. If the positivity depends on the localization of a particular antigen, various other factors, such as the condition of the tissue, the homogeneity of the fixation and the possible artefacts, can play an important role in an accurate interpretation.

In the ERα analysis, to avoid misleading, in the ten and four of the examined specimens there were positive (nuclear and cytoplasmatic) sebaceous lobules and/or hair follicles (basal cells of infundibulum and isthmus), respectively, and they served as the internal positive control. Conversely, breast carcinoma samples served as the external positive control.

Two cases have been molecularly characterized at our laboratory of oncologic and transplantation molecular pathology. DNA extraction and mutation analyses in oncogenes BRAF, KIT and NRAS with Sanger direct sequencing has been performed as previously described[144].

3.7 Dermatoscopy evaluation

The dermatoscopic evaluation of the select patients was performed by two expert dermatologists trained in dermoscopy. All the examined imagines were collected at the Melanoma Unit of the University of Bologna. The device used for obtaining both clinical and dermatoscopic images was FotoFinder Medicam® 800HD Dermoscope (Fotofinder Systems GmbH, Birbach, Germany), with a 1920 x 1080 resolution. Both polarized and non-polarized light was used for the pictures acquisition. Written consent was obtained by all patients for the use of images for research purpose. The dermatoscopic patterns analysed were atypical network, blue-white veil, atypical vascular pattern, irregular streaks, irregular dots/globules, irregular blotches, regression structures, hyperpigmentation, distribution of pigment.

4.0 Results

4.1 Epidemiological analysis

We reviewed our database encompasses all melanoma patients evaluated at the Melanoma Unit at the University of Bologna, Italy from 2008 to 2018, finding 1054 females. From these, a total of 810 women with a previous diagnosis of CM met the inclusion criteria and were initially included in the cohort study. The main characteristics of the individuals and the CM were summarized in Table 1 and 2.

The median age at menarche was 12,16 in our population study; meanwhile, the average value of menopausal age was 52,47. The hormone therapy duration, including with this term, both OCPs and HRT, was 7,37 years. The mean value of pregnancies and abortions were respectively 1,25 and 0,36 with age at first pregnancy of 30,78 years. Of the available data, almost 30% reported a positive family history of CM and a total of 67 patients (6,91%) had a multiple primary CM. The females who had melanoma during pregnancy were 9 (1,1%), besides the cases of ovarian stimulation, as part of ARTs, were 6 in total (0,7%). Finally, women with a medical history of breast cancer were 24 (2,9%).

Regarding CM features, the most common location was the trunk and the legs with an identical percentage (almost 27%), following by arms (13,46%), head and neck (7,53%), acral location (4,20%) and finally the external mucosal site with a total of 3 cases (0,37%) in all our cohort. The majority of CM were thin melanomas with Breslow thickness (BT) less or equal to 1 mm (80,62%). The thicker CMs (≥ 2 mm, pT3 according to the 8th edition of the AJCC Cancer Staging Manual) represented just the 9,38% of all the cases. High mitotic index and ulceration were found in less than 8% of patients. The most common histotype was superficial spreading melanoma (ssm) (69,14%), followed by nodular melanoma (nm) (7,04%) and acral lentiginous melanoma (alm) (3,70%). A vertical cancer growth was identified in more than a quarter (25,56%) of the subjects. The TIL was absent in slightly more than half of the subjects (51,36%). Among the others, the brisk TIL was predominant (33,21%). Lymphovascular invasion and extensive regression (>75%) was respectively detected in about 3% and 4% of individuals.

Considering the mean age at menopause in our study population, in order to investigate differences between women in different physiological hormonal status, we divided the population study into two main subgroups: women aged over 52 and women having less than 52 years old. The two groups were both of 405 individuals, precisely 50% of our cohort. Table 3 displays a summary of the tumour characteristics distributed by age group. The location was significant divergent among the two subunits: in particular women in fertile age are more prone to have CM on the trunk. Conversely, females on the postmenopausal period are more likely to develop CM on arms, legs and acral location (Figure 2). The Breslow thickness was statistically (P-value <0,001) higher in women aged over 52 years, compared with the younger ones (Figure 3). Even if no statistical significance was observed regarding the mitotic index, a greater percentage (11,46% versus 6,16%) of postmenopausal females had a high (≥3) mitotic index (Figure 4). Furthermore, a statistical

relevance (Figure 5) is noticed about CM ulceration, most common in postmenopausal age. Regarding the histotype not statistically meaningful was found, although older females are more likely to develop nodular melanoma (10,32% versus 6,67 %) (Figure 6).

Due to the insufficient number of cases, a statistical comparison between women who underwent to ovarian stimulation (a total of 6 persons) and the other women of the cohort, was not performed. The individual and cancer characteristics of females experienced ARTs are summarized together with immunohistochemical analysis in Table 6.

Concerning the group for women with a history of BC, when compared with the women without BC, we observed a significantly higher percentage of CM with "non-brisk" TILs (Figure 7).

4.2 Histological and Immunohistochemical analysis of selected cases

From a total of 30 selected cases with FFPE blocks accessible at the Dermatopathology unit of the University of Bologna, two patients, belonging to the control groups, were excluded from the analysis due to scarce biological material available. A total of 28 cases were analysed. According to the 2018 WHO Classification of Skin Tumour, the histological subtypes were 24 (85.7%) superficial spreading melanoma (SSM), 3 (10.7%) naevoid melanoma (NEM) and 1 (3.6%) acral lentiginous melanoma (ALM). The Breslow thickness ranged from 0 to 1.9 mm (mean: 0.69 mm). According to the 8th edition of the AJCC Cancer Staging Manual, the pT stages were 2 (7.1%) pTis, 17 (60.7%) pT1a, 2 (7.1%) pT1b, 6 (21.3%) pT2a, 1 (3.8%) pT2b. Sentinel lymph node biopsy was performed by nine patients (32,1%), in one case subclinical micrometastasis was discovered, and the patient underwent to radical lymphadenectomy, with no disease relapses at three years of follow-up. None of the investigated patients had distant metastasis from CM. The mean value of follow-up was four years (range: 2-10). No death from melanoma or other causes occurred.

All melanoma cases showed absent or focal nuclear positivity for ERα (21/28 cases with score 0; 7/28 cases with focal nuclear staining, range: 1-3%). The subjects with slight nuclear presence were three controls, three patients with antioestrogen therapy for BC and one woman with previous ARTs. At the opposite, 12/28 cases showed score 0 for cytoplasmatic ERα, whereas score 1 and 2 was both observed in 16/28 patients (Figure 8). The median value for cytoplasmatic ERα was 30.5%, with a median value of 25% and a range of 0-80%. The staining had a granular-like aspect. All cases with BC had a cytoplasmatic ERα positivity, except one. She was not under antioestrogen therapy at the time of CM onset, and recently, she has developed a third tumour: a clear cell renal cell carcinoma. A genetic mutation screening is actually in progress.

About ERβ, positive nuclear expression (score 1 or 2) was detected just in 11/28 subjects, six belonging to the BC group, four controls and one with ovarian stimulation for infertility. Negative presence of ERβ receptor characterized the majority of our patients, 17/28 cases. The nuclear staining had a wide range of

values (from 0 to 90%) (Figure 9). On the other hand, almost all of the examined melanomas 22/28 displayed a variable cytoplasmatic expression of ERβ (mean value: 59.8%).

All the patient's characteristics and immunohistochemical results divided by groups are listed in Table 5 and 6.

Upon the Pearson correlation analysis, no association was shown between cytoplasmatic ERα and age or Breslow thickness. No statistical correlation was either found between nuclear ERβ and age. Regarding nuclear ERβ and Breslow thickness, the result was near to achieve the significance, although it did not reach any. (Figure 10)

No association emerged from ERs immunohistochemical expression and other histopathological such as regression, mitotic index or other histological parameters of CM.

The two cases with molecular characterization belong to the women who had ovarian stimulation, and both showed BRAF V600E mutation.

4.3 Dermatoscopic aspects

The dermatoscopic analysis was performed for all 28 patients. All patients belonged to phototype II according to Fitzpatrick classification. In the total of women with previous BC and ARTs, CM was characterized by a marked, dark brown and black atypical network and displayed irregular blotches (100%). Moreover, the presence of irregular streaks was common (71,4%) (Figure 11). Rarely the CMs in this group had an irregular pigmented distribution (28,6%) or an atypical vascular pattern (35,7%). Furthermore, generally, the atypical network was present in 89,3% of all subjects, although, in the control group, the network was less pigmented and irregularly distributed (78,57%). Finally, irregular dots/globules characterized the majority of the controls (92,8%) (Figure 12).

5.0 Discussion

The evidence clearly shows that women have a better outcome for many cancer types, including melanoma. Gender disparities in CM are present worldwide and over time despite dissimilar behaviours and latitudes. Furthermore, the female melanoma prognosis is still better than men when adjusting for the known melanoma prognostic factors[145]. These data, reported in the literature, strongly suggest that in addition to cancer-specific characteristics, an important role is played by the host's gender. As well known, the incidence of CM has higher during fertile age, conversely is lower and associated with a poorer prognosis in menopausal era[146]. Our cohort retrospective aimed to study female CM, considering both endogenous and exogenous oestrogen sources. Endogenous hormone basis was investigated through epidemiological studies examining the differences between women in fertile age and postmenopausal period, using as a cut-off the mean value of age at menopause. From this first analysis, we found a divergence in CM location: among younger women, CM are more likely to develop in the trunk, on contrary postmenopausal females are more

prone to have CM on extremities. The same result was already reported in a large population-based cohort study of 1347 Norwegian women. In their work, Støer NC et al[56] assess that this heterogeneity is probably caused by confounding factors as age, as CMs on the trunk are more common in younger people, mostly young women. Melanomas developing at different body sites are associated with distinct patterns of sun exposure. Whiteman DC et al. in 2006 [147] stated that trunk melanomas are more likely associated with intermittent patterns of sun exposure, supporting his hypothesis of divergent causal pathways in CM[148]. The two CM paths should be related respectively to chronic sun exposure and the presence of few naevi and to intense-intermitted solar exposure and higher mole density with a freckling tendency and a melanocyte instability[149]. In addition, regarding UV radiation, it should also be considered the role that estrogens play in modulating melanogenesis and decreasing oxidative stress as demonstrated in many in vitro studies[150,151]. Thick melanomas, ulceration and high (≥3) mitotic index was seen in older women. This more aggressive histopathological profile of CM is principally due to a selection bias and other confounding factors, such as histotype. Although, an influence of lower oestrogens level in down-regulation of immune processes cannot be totally excluded[152]. Moreover, it was observed that nodular melanoma is more often found in postmenopausal women, and this fact is confirmed in literature by the reflection of its higher incidence directly related to elder status among patients[153].

According to the investigation, we intend to realize, in our cohort study, we observed two different opposite situations concerning exogenous oestrogen sources.

One was represented by women who underwent ovarian stimulation for ARTs, and the other one was observed among women following antioestrogen therapy for breast cancer.

The first group observed was a tiny percentage (0,7%) compared to the total number of investigated cases. For this reason, a statistical analysis was not performed. Three of those subjects was treat by follitropin Alfa injection to stimulate ovarian follicular development. This drug was reported to have induced in one case of recurrent malignant melanoma[154]. Nevertheless, there is no proven evidence that there is a higher melanoma risk after assisted reproduction[95]

In the second group, there were a higher number of patients investigated, though the percentage was still not high (2,9%). On those patients, a statistical analysis was performed to have a comparison between the rest of the investigated cases. The emerging results lead to a statistical significance regarding the presence of "non-brisk" TILs. TIL has been considered in several studies that have contradictory results on its meaning in the CM prognosis. A recent study by Sinnamon AJ et al[155] showed that TIL is a predictive factor of sentinel lymph node positivity among men, but no association was found in women. In our study, an association between the non-brisk pattern and the group of women with previous BC was observed. According to our finding, further investigations must be needed to evaluate whether different cancers on the same individual can have a similar TILs composition or not. It could be necessary to examine also TILs meaning related to disease prognosis and patients' treatment response[156].

In addition to the epidemiological investigation we have discussed, it was also performed immunohistochemical analysis to evaluate the presence of ERα and ERβ among melanoma cells of the women undergone ovarian stimulation for ARTs and of the women following antioestrogen therapy for breast cancer. Results emerging from this analysis show that all women assuming aromatase inhibitors therapy have a widespread cytoplasmatic expression of ERα in melanoma cells. In a previous study of our group, we found a similar result in women with nodular melanoma treated with multiple cycles of hormonal stimulation for in vitro fertilization[5]. The meaning of cytoplasmatic ERα in melanoma is still debated. It could suggest a potentially significant role of oestrogen non-genomic pathway in these patients[157], or it can be a mechanism of ERs modulation in response to aromatase inhibitor therapy[158]. Finally, the reason of its presence in a granular shape can be just the evidence of a false positive due to the residual endogen peroxidases, even if endogen peroxidases were accurately inhibited following our protocol for immunohistochemical staining. Concerning ERβ, our study shows no correlation between nuclear positivity and Breslow thickness values, contrarily to what was displayed in literature. Undoubtedly the use of different anti-ERβ antibodies can partially affect the immunohistochemical analysis. In our study, we used an antibody directed towards isoform 1, that is favoured in many studies[159]. Eventually, a dermatoscopic analysis was performed on all patients selected for immunohistochemical analysis to investigate the clinical aspect of their CMs. As it was shown in literature by Auriemma et al. [160], our work also evidences, among the two particular patients subgroups a darker brown atypical network and irregular blotches that clinically characterized the CM in comparison to control groups.

Our investigation is limited by a number of factors, first including the small amount of available cases. For these reasons, it can be subjected to many biases. However, our work tried to enlighten some of the existing shadows on the role of ERs and hormonal factors in CM in a real-world setting. Based upon our direct experience, confirmed by literature, it can be affirmed that ERs and hormonal factors play a marginal role in the aetiology of female melanoma. Instead, sex-specific genetics and immunity have a leading effect on melanoma genesis, development and progression[161].

6.0 Present and future perspective

Drugs target ERs are still under investigation as a therapy for CM, despite tamoxifen (TAM), a non-selective ER antagonist, was first observed, in clinical trials, to have no relevant effect on CM survival rate[162].

This finding is probably due to TAM proprieties: at the same time antitumoral and pro-survival agent, depending on ERα/ERβ ratio in the target tissue[163]. Moreover, TAM has also demonstrated an agonist action towards GPER in vitro[124] It is recently showed that endoxifen, a 100 times more powerful active metabolite of TAM, can be a promising new therapeutic agent against CM by blocking ER transcription[164]. Besides, selective ERβ agonist, LY500307, has been observed to have efficacy against lung metastasis from CM, both in vitro and in vivo. It acts in increasing the innate immunity's ability to suppress cancer through the production of IL-

1β. This latter is a chemotactic molecule produced by cancer cells after the stimulation of their ERβs. This molecular mechanism is another example of the primary role that estrogen signalling can play in CM and how much it can be significant for future anticancer pharmacological research[165]. GPER also has a relevant part in immunomodulation operated by estrogen signalling. In 2018 Natale A. et al.[166] proved that GPER action prevents tumour spreading and selects a cancer immune profile that makes CM more vulnerable to immune checkpoint blockade agents.

Finally, an emerging research field is represented by the connections between microRNAs (miRNAs) and estrogen receptor pathways.

Several studies found that miRNAs dysregulation/ specific disease profiles are common in cancer cells, and they are implicated in tumour development, progression but also cancer suppression[167,168]. In particular, miRNAs expression has demonstrated a regulatory effect on ERs expression. Specifically, miR-206, miR-22 and let-7 have demonstrated to downregulate ERα levels in breast cancer, with a consequent proapoptotic and antiproliferative effect [169-171]. Besides estrogen-induced miR-196a expression has a prognostic value in hormone therapy, responsive breast cancer[172]. On the other hand, ERα-mediated estrogen signalling downregulates the level of miR-21, miR-26a, miR-140, miR-181b and overexpress miR-206, and miR-190a, miR-191, miR-203 and miR-425[173]. Regarding ERβ, less is known. Several studies show that it is closely related to suppression in miR-17, miR-30a, miR-200a and miR-200b expression, and it upregulates of miR-23b, miR-24-1 and miR-27b[174]. Recently also some transcriptional factors have been discovered to recognize the DNA ERE-elements and activate miRNAs transcription depending on ERs action[175]. The interaction between tissue specificity of miRNA expression and ERs and GPER is even more complex and still under investigation also in endothelial cells[176].

In melanoma, a key role in disease spreading and progression has been proposed for miR-221 and -222 expression, negatively regulated by ERα. Furthermore, miR-221/222 modulate directly KIT oncogene that is typically mutated in acral and mucosal melanoma[177,178].

More is to investigate in the composite scenario of ERs, GPER, miRNA expression and their effect on the melanoma microenvironment.

7.0 Tables

Table 1 and Table 2 summarizes, respectively, individual and tumour characteristics.

Table 1. Individual characteristics.

Age at diagnosis, years, n (%)	
≤52	405 (50.00)
>52	405 (50.00)
Phototype, n(%)	
1	50 (6.17)
2	548 (67.65)
3	212 (26.17)
Photodamage, n(%)	
No	191 (23.59)
Yes	619 (76.41)
Familiarity, n(%)	
No	578 (71.35)
Yes	232 (28.65)
Other cutaneous tumours, n(%)	
No	386 (63.69)
Yes	220 (36.30)
NA	204
Previous breast cancer, n(%)	
No	786 (97.03)
Yes	24 (2.96)
Age at menarche, years, mean (sd)	12,16
Assisted procreation, n(%)	
No	804 (99.25)
Yes	6 (0.74)
Hormone therapy, n(%)	
No	183 (36.38)
Yes	320 (63.62)
NA	307
Hormone therapy duration, years, mean (sd)	7.37 (5.86)
Number of pregnancies, mean (SD)	1.25 (0.97)
Age at first pregnancy, years, mean (SD)	30.78 (6.41)
Number of abortions, mean (SD)	0.36 (0.76)
Age at menopause, years, mean (SD)	52.47 (3.25)

Table 2. Tumour characteristics.

Location, n(%)	
Head / neck	60 (7.53)
Trunk	221 (27.28)
Arms	109 (13.46)
Legs	219 (27.04)

Acrals	34 (4.20)
Mucous membranes	3 (0.37)
NA	164 (20.25)
Tumour thickness, mm, mean (sd)	
≤1	653 (80.62)
(1,2]	76 (9.38)
>2	76 (9.38)
NA	5 (0.62)
Mitotic index, mean (sd)	
0	497 (61.36)
1-2	132 (16.29)
≥3	61 (7.53)
NA	120 (14.81)
Ulceration, n(%)	
No	673 (83.09)
Yes	64 (7.90)
NA	73 (9.01)
Histotype, n(%)	
ssm	560 (69.14)
nm	57 (7.04)
lm	9 (1.11)
lmm	8 (0.99)
alm	30 (3.70)
desmoplastic	1 (0.12)
rare type	4 (0.49)
NA	141 (17.41)
Type of growth, n(%)	
Radial	476 (58.77)
Vertical	207 (25.56)
NA	127 (15.67)
Satellite nodules, n(%)	
No	620 (76.54)
Yes	8 (0.99)
NA	182 (22.47)
TIL, n(%)	
No	416 (51.36)
Brisk	269 (33.21)
Non-brisk	112 (13.83)
NA	13 (1.61)
Lymphovascular invasion, n(%)	
No	636 (78.52)
Yes	26 (3.21)
NA	148 (18.27)
Regression, n(%)	
No	408 (50.37)
Yes	236 (29.14)
>75%	33 (4.07)
NA	133 (16.42)
Metastases, n(%)	
No	585 (72.22)
Yes	100 (12.35)
NA	125 (15.43)

Table 3. Tumour characteristics by age group. Significant p-values are in bold.

	Age ≤ 52	Age > 52	P-value
Location, n(%)			**<0.001**
Head / neck	10 (3.15)	50 (15.20)	
Trunk	137 (43.22)	84 (25.53)	
Arms	49 (15.46)	60 (18.24)	
Legs	107 (33.75)	112 (34.04)	
Acrals	13 (4.10)	21 (6.38)	
Mucous membranes	1 (0.32)	2 (0.61)	
Tumour thickness, mm, mean (sd)			**<0.001**
≤1	338 (83.87)	315 (78.36)	
(1,2]	43 (10.67)	33 (8.21)	
>2	22 (5.46)	54 (13.43)	
Mitotic index, mean (sd)			**0.040**
0	250 (73.31)	247 (70.77)	
1-2	70 (20.53)	62 (17.77)	
≥3	21 (6.16)	40 (11.46)	
Ulceration, n(%)			**0.001**
No	347 (94.81)	326 (87.87)	
Yes	19 (5.19)	45 (12.13)	
Histotype, n(%)			**0.037**
ssm	290 (87.88)	270 (79.65)	
nm	22 (6.67)	35 (10.32)	
lm	2 (0.61)	7 (2.06)	
lmm	1 (0.30)	7 (2.06)	
alm	13 (3.94)	17 (5.01)	
desmoplastic	0 (0.00)	1 (0.29)	
rare type	2 (0.61)	2 (0.59)	
Type of growth, n(%)			0.740
Radial	232 (69.05)	244 (70.32)	
Vertical	104 (30.95)	103 (29.68)	
Satellite nodules, n(%)			0.287
No	304 (99.35)	316 (98.14)	
Yes	2 (0.65)	6 (1.86)	
TIL, n(%)			0.920
No	206 (51.76)	210 (52.63)	
Brisk	137 (34.42)	132 (33.08)	
Non-brisk	55 (13.82)	57 (14.29)	
Invasion, n(%)			0.070
No	321 (97.57)	315 (94.59)	
Yes	8 (2.43)	18 (5.41)	
Regression, n(%)			0.382
No	197 (58.81)	211 (61.70)	
Yes	118 (35.22)	118 (34.50)	
>75%	20 (5.97)	13 (3.80)	
Metastases, n(%)			0.786
No	443 (75.73)	410 (70.09)	
Yes	142 (24.27)	175 (29.91)	

Table 4. Tumour characteristics by the presence of breast cancer. Significant p-values are in bold
.

	No breast cancer	Breast Cancer	P-value
Location, n(%)			0.142
Head / neck	59 (9.41)	2 (8.33)	
Trunk	210 (33.49)	12 (50.00)	
Arms	105 (16.75)	5 (20.83)	
Legs	217 (34.61)	3 (12.50)	
Acrals	33 (5.27)	2 (8.33)	
Mucous membranes	3 (0.48)	0 (0.00)	
Tumour thickness, mm, mean (SD)			0.507
≤1	566 (74.73)	19 (79.17)	
(1,2]	95 (12.58)	4 (16.67)	
>2	96 (12.69)	1 (4.17)	
Mitotic index, mean (sd)			0.272
0	480 (71.64)	19 (79.17)	
1-2	131 (19.55)	2 (8.33)	
≥3	59 (8.21)	3 (12.50)	
Ulceration, n(%)			1.000
No	654 (91.21)	22 (91.67)	
Yes	63 (8.79)	2 (8.33)	
Histotype, n(%)			0.940
ssm	538 (83.41)	22 (91.67)	
nm	55 (8.53)	2 (8.33)	
lm	9 (1.40)	0 (0.00)	
lmm	8 (1.24)	0 (0.00)	
alm	30 (4.65)	0 (0.00)	
desmoplastic	1 (0.15)	0 (0.00)	
rare type	4 (0.62)	0 (0.00)	
Type of growth, n(%)			0.213
Radial	456 (69.20)	20 (83.33)	
Vertical	203 (30.80)	4 (17.67)	
Satellite nodules, n(%)			1.000
No	606 (98.70)	24 (100.00)	
Yes	8 (1.30)	0 (0.00)	
TIL, n(%)			**0.001**
No	406 (52.52)	10 (41.67)	
Brisk	265 (34.28)	4 (16.67)	
Non-brisk	102 (13.20)	10 (41.67)	
Invasion, n(%)			1.000
No	612 (94.44)	24 (100.00)	
Yes	36 (5.56)	0 (0.00)	
Regression, n(%)			0.486
No	395 (60.49)	13 (54.17)	
Yes	225 (34.46)	11 (45.83)	
>75%	33 (5.05)	0 (0.00)	
Metastases, n(%)			0.330
No	565 (85.48)	20 (83.33)	
Yes	96 (14.52)	4 (16.67)	

Table 5. Clinical, histological and immunohistochemical data of patients with breast cancer included in the study.

Patient number	Age at melanoma diagnosis	Age at breast cancer diagnosis	Drug therapy for breast cancer	Duration of breast cancer therapy (years)	Photodamaging	Number of nevi	Melanoma location (0=extremities, 1=trunk and dorsum, 2=head neck, 3=other)	Melanoma histotype (0=superficial spreading melanoma, 1=naevoid melanoma)	Breslow thickness	ERα N in %	ERα N (0<20%, 1=21%-50%, 2>50%)	ERα C in %	ERα C (0<20%, 1=21%-50%, 2>50%)	ERβ N in %	ERβ N (0<20%, 1=21%-50%, 2>50%)	ERβ C in %
1	68	67	Aromatase inhibitor (Anastrozole)	1	yes	50-100	0	0	0.4	2	0	55	2	60	2	90
2	65	64	Aromatase inhibitor (Anastrozole)	1	yes	<50	1	1	0.6	2	0	60	2	80	2	80
3	63	62	Aromatase inhibitor (Anastrozole)	1	yes	<50	1	0	0.3	0	0	55	2	80	2	20
4	48	50	Aromatase inhibitor (Letrozole)	2	No	20-30	1	0	0.2	0	0	25	1	5	0	20
5	44	52	Trastuzumab and aromatase inhibitor (Anatrozole)	1	No	<50	0	0	0	0	0	60	2	80	2	80
6	58	49	Interferon at CM diagnosis, then letrozole	1	Yes	5	1	0	0,2	0	0	5	0	10	0	70
7	51	53	Aromatase inhibitor (Lestrozole)	2	No	50-100	1	0	1,3	2	0	70	2	10	0	90
8	81	81	Aromatase inhibitor (Anastrozole)	<1	yes	<20	2	0	0.6	0	0	80	2	60	2	90
9	63	62	Aromatase inhibitor (Anastrozole)	1	yes	30	1	0	0.3	0	0	40	1	60	2	60

Table 6. Clinical, histological and immunohistochemical data of patients with ovarian stimulation included in the study.

Patient number	Age at melanoma diagnosis	Age at ovarian stimulation	Drug therapy for ovarian stimulation	Cycles of ovarian stimulation	Successful pregnancy (yes/no)	Photodamaging	Number of nevi	Melanoma location (0=extremities, 1=trunk and dorsum, 2=head neck, 3=other)	Melanoma histotype (0=superficial spreading melanoma, 1=naevoid melanoma 2= acral melanoma)	Breslow thickness	ERα N in %	ERα N (0<20%, 1=21%-50%, 2>50%)	ERα C in %	ERα C (0<20%, 1=21%-50%, 2>50%)	ERβ N in %	ERβ N (0<20%, 1=21%-50%, 2>50%)	ERβ C in %
10	39	38	Progesterone, others unknown	1	yes	no	30-50	1	1	1.2	0	0	10	0	0	0	20
11	48	40	Unknown	1	yes	yes	<10	1	0	0.2	0	0	25	1	5	0	20
12	43	34	Uncomplete ovarian stimulation	1	yes	yes	<20	0	2	1	0	0	5	0	5	0	55
13	34		Follitropin alfa, triptorelin acetate injection, menotropins, ganirelix	3	no	yes	>50	0	0	1.2	2	0	30	1	5	0	100
14	43		Follitropin alfa, ganirelix, chorionic gonadotropin, progesterone	1	yes	yes	>50	0	1	1.5	0	0	15	0	90	2	30
15	30	28	Follitropin alfa, ganirelix, chorionic gonadotropin, progesterone	2	no	yes	50-100	1	0	0.6	0	0	0	0	0	0	70

Table 7. Dermatoscopic features of CM in patients with ovarian stimulation and antioestrogens cancer therapy versus control groups

Dermoscopic features	Cases	Controls
Atypical pigmented network	14/14 (100%)	12/14 (85,7%)
Blue-white veil	7/14 (50%)	2/14 (14,3%)
Atypical vascular pattern	5/14 (35,7%)	7/14 (50%)
Irregular streaks	10/14 (71,4%)	9/14 (64,3%)
Irregular dots/globules	8/14 (57,1%)	13/14 (92,8%)
Irregular blotches	14/14 (100%)	7/14 (50%)
Regression structures	7/14 (50%)	8/14 (57,1%)
Irregular pigment distribution	4/14 (28,6%)	11/14 (78,57%)
Pigmentation in more than 50% of the CM	9/14 (64,3%)	6/14 (42,8%)

8.0 Figures

Figure 1 Molecular structures of estrogen receptors

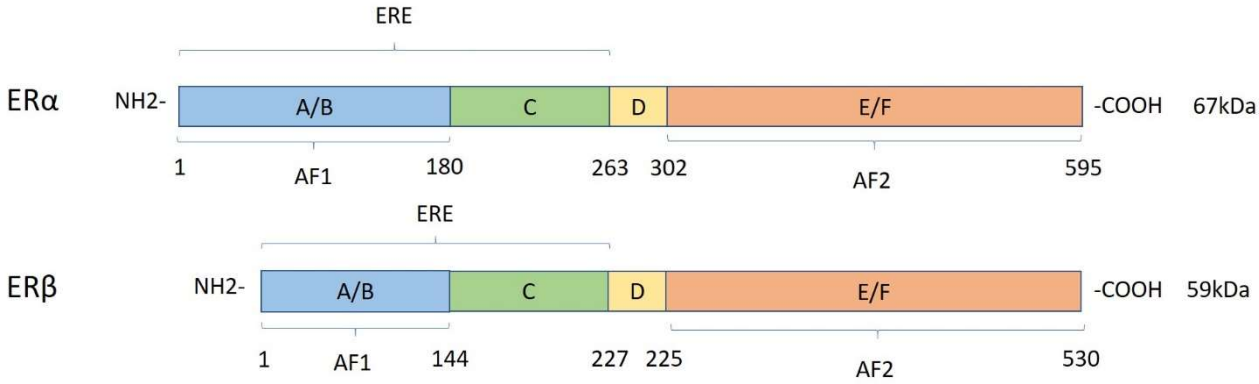


Figure 2. Location distribution by age group.

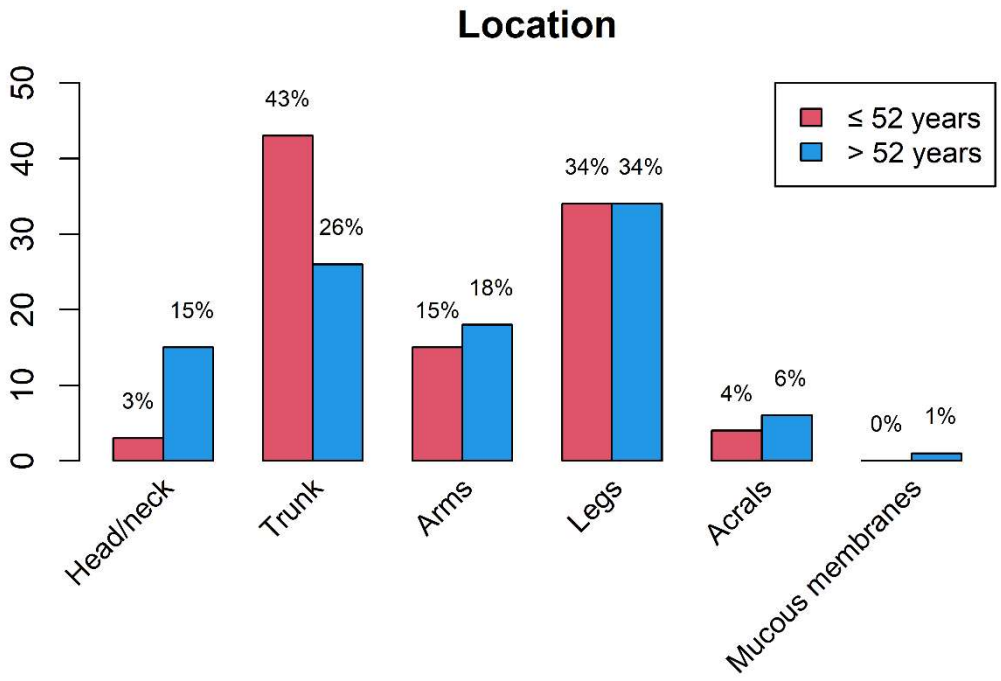


Figure 3. Breslow thickness distribution by age group.

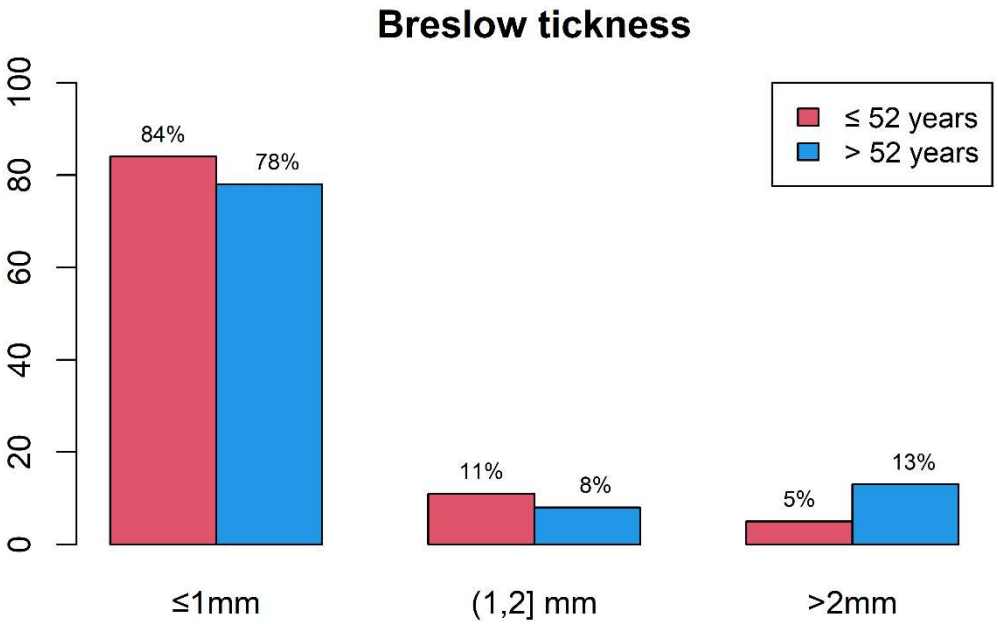


Figure 4. Mitotic index distribution by age group.

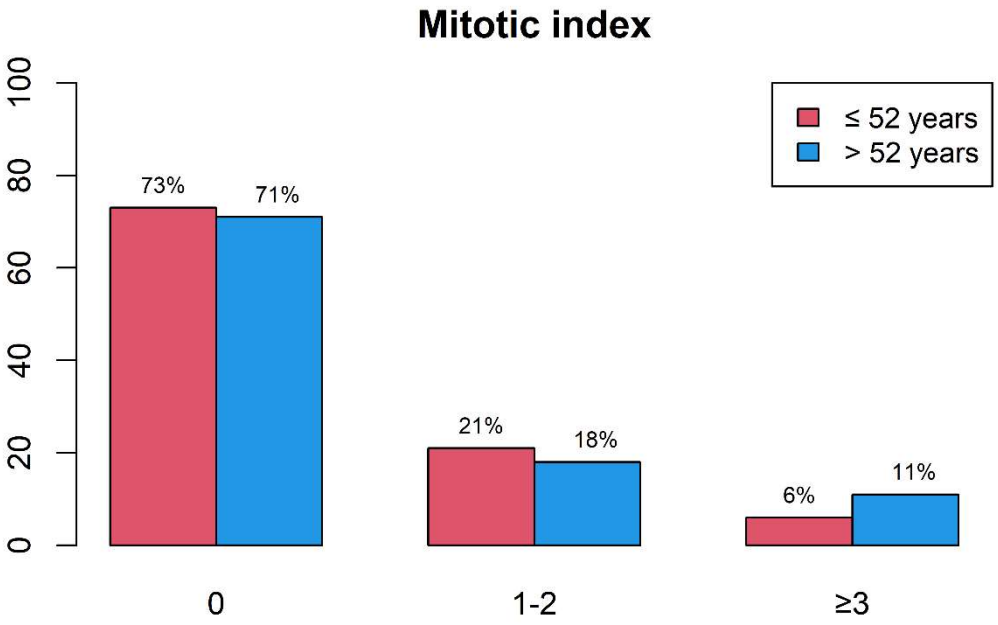


Figure 5. Ulceration distribution by age group.

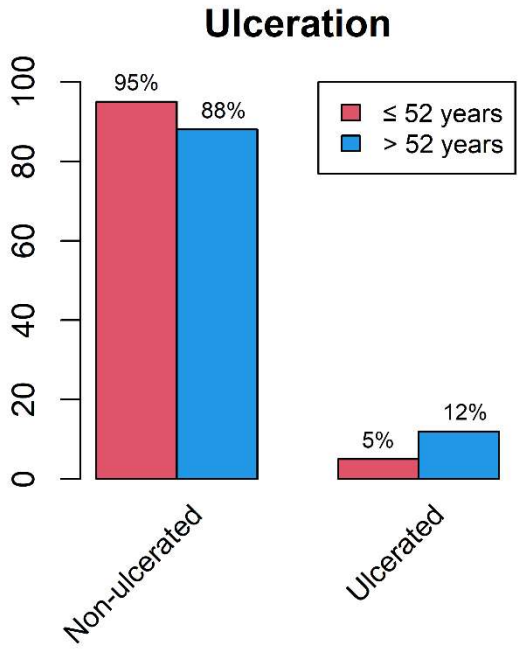


Figure 6. Histotype distribution by age group.

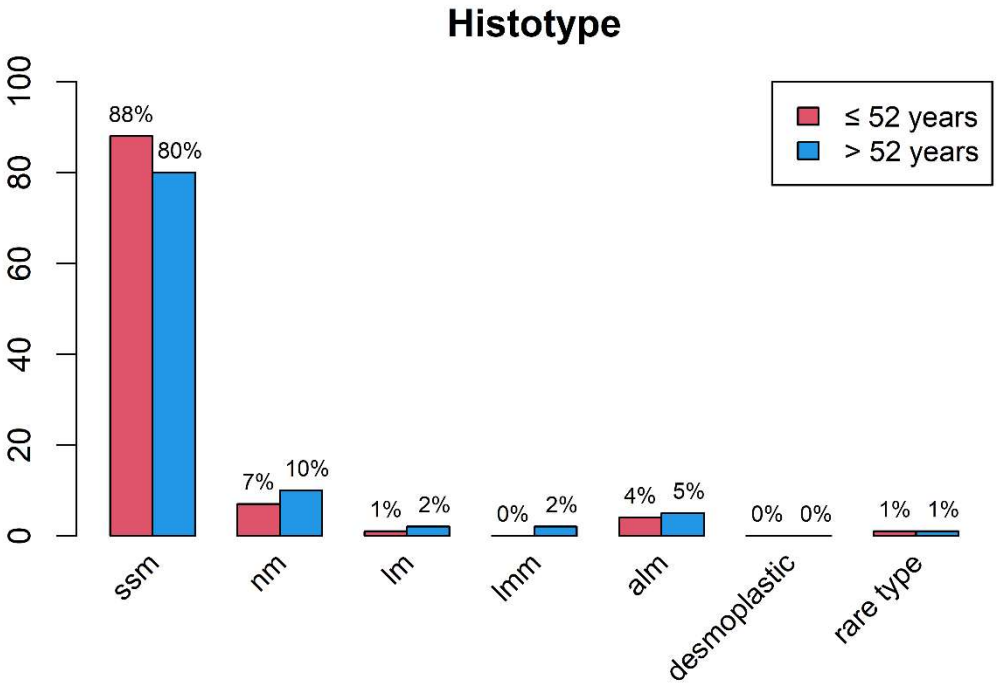


Figure 7. TIL distribution by the presence of breast cancer.

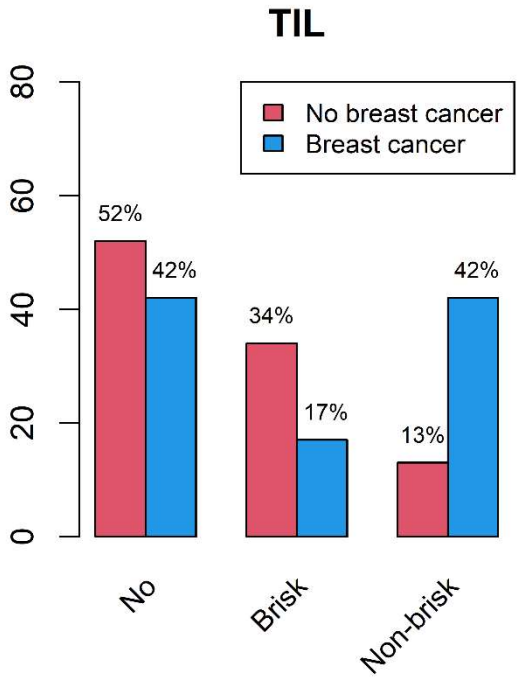


Figure 8. Diffuse cytoplasmatic positivity for ERα in the melanoma cells (original magnification ×100)

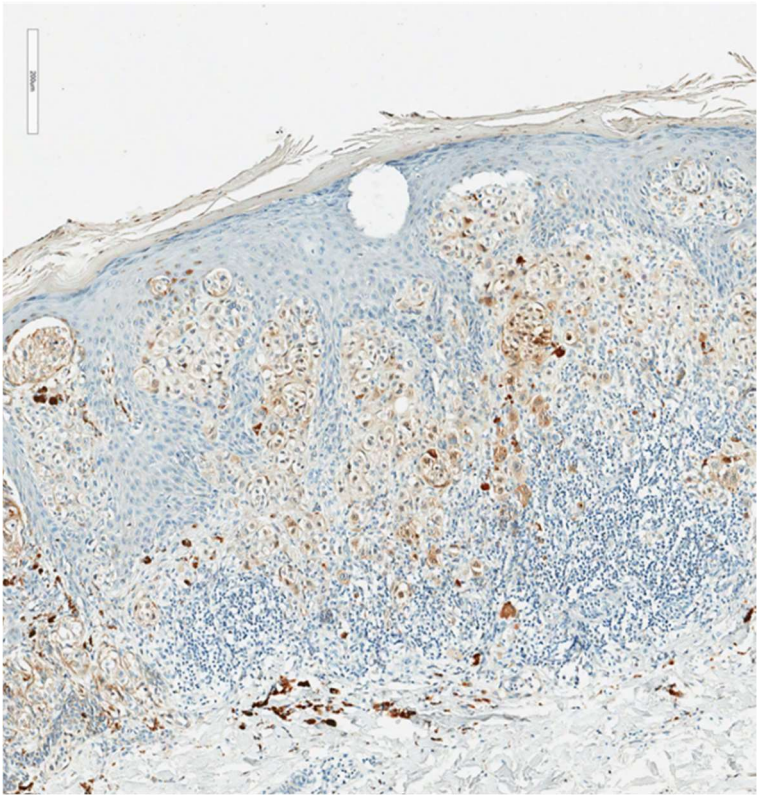


Figure 9. Nuclear positivity for ERβ in the melanoma cells of the same case (original magnification ×100)

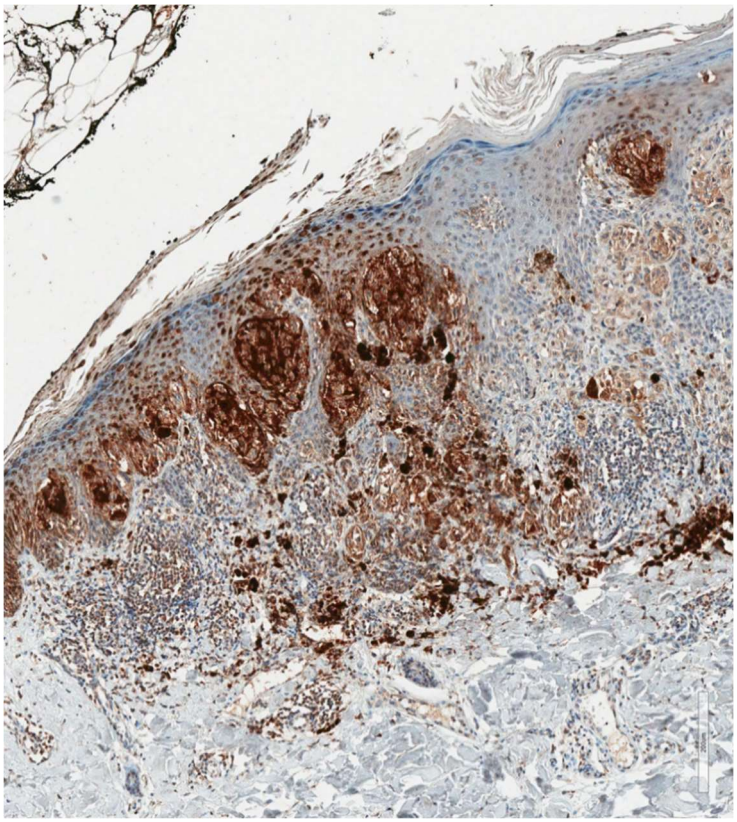


Figure 10. The scatter plot of the data, indicating the reasonableness of assuming a linear association between the variables.

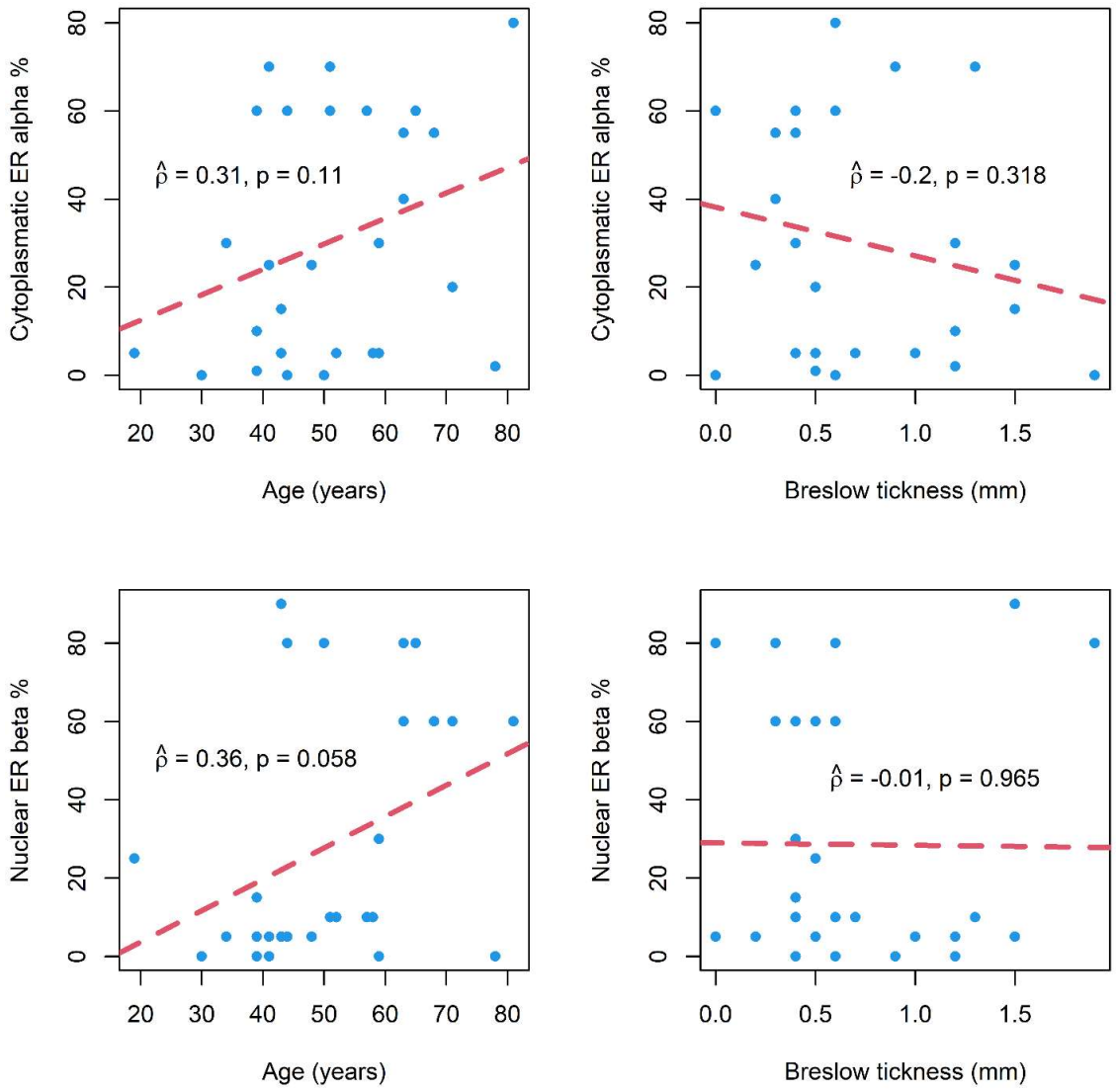


Figure 11. Cutaneous melanoma of the trunk in a patient underwent to assisted reproductive treatment

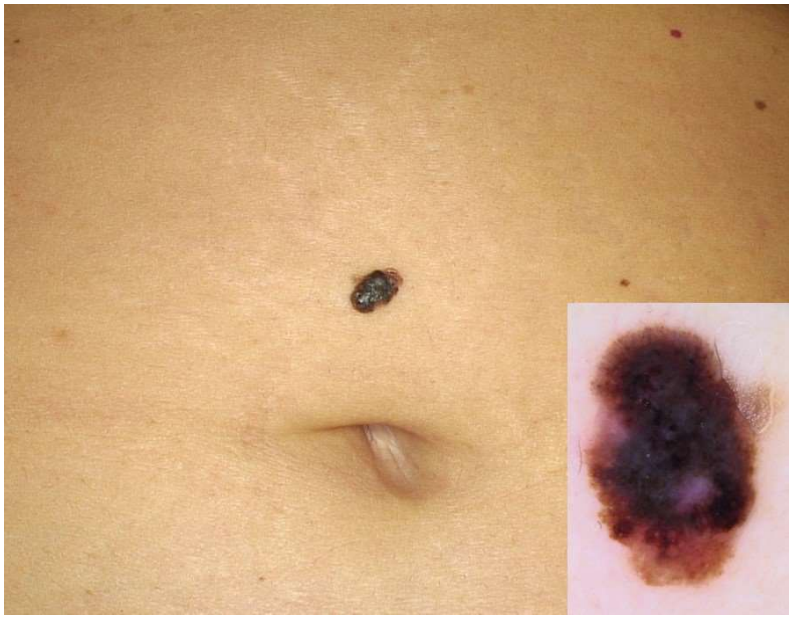
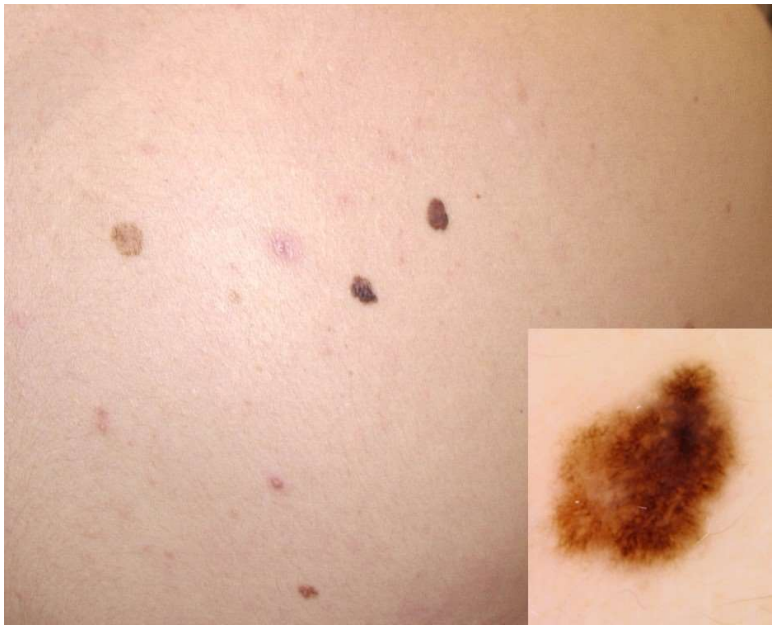


Figure 12. Cutaneous melanoma of the trunk in a patient belonging to the control group



References

1. Sacchetto L, Zanetti R, Comber H, et al. Trends in incidence of thick, thin and in situ melanoma in Europe. Eur J Cancer. 2018; 92:108-118.
2. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer 2013; 49:1374–403.
3. AIOM and AIRTUM Working Group I numeri del cancro in Italia, 2019.
4. Olsen CM. Reproductive factors and risk of melanoma: still unresolved. Br J Dermatol. 2019 Aug;181(2):239. doi: 10.1111/bjd.18136. Epub 2019 Jul 9. PMID: 31290145.
5. Lens M, Bataille V. Melanoma in relation to reproductive and hormonal factors in women: current review on controversial issues. Cancer Causes Control. 2008;19(5):437-442. doi:10.1007/s10552-008-9110-4
6. Han MA, Storman D, Al-Rammahy H, et al. Impact of maternal reproductive factors on cancer risks of offspring: A systematic review and meta-analysis of cohort studies. PLoS One. 2020;15(3):e0230721
7. Folkerd EJ, Dowsett M. Influence of sex hormones on cancer progression. J Clin Oncol. 2010;28(26):4038-4044. doi:10.1200/JCO.2009.27.4290
8. Hartman J, Ström A, Gustafsson JA. Estrogen receptor beta in breast cancer—diagnostic implications. Steroids. 2009;74(8):635-41.
9. Hartman J, Edvardsson K, Lindberg K, et al. Tumor repressive functions of estrogen receptor beta in SW480 colon cancer cells. Cancer Res.2009;69(15):6100-6.
10. Drummond AE, Fuller PJ. The importance of ERbeta signalling in the ovary. J Endocrinol. 2010;205(1):15-23
11. Thomas CG, Ström A, Lindberg K, Gustafsson JA. Estrogen receptor beta decreases survival of p53-defective cancer cells after DNA damage by impairing G(2)/M checkpoint signaling. Breast Cancer Res. Treat. 2010;
12. Lindberg K, Ström A, Lock JG, Gustafsson JA, Haldosén LA, Helguero LA. Expression of estrogen receptor beta increases integrin alpha1 and integrin beta1 levels and enhances adhesion of breast cancer cells. J. Cell. Physiol. 2010;222(1):156-67
13. Schmidt AN, Nanney LB, Boyd AS, King LE Jr, Ellis DL. Oestrogen receptor beta expression in melanocytic lesions. Exp. Dermatol. 2006; 15:971-80
14. De Giorgi V, Mavilia C, Massi D, et al. Estrogen receptor expression in cutaneous melanoma. Arch. Dermatol. 2009;145(1):30-3.
15. Ohata C, Tadokoro T, Itami S. Expression of estrogen receptor β in normal skin, melanocytic nevi and malignant melanoma. J. Dermatol. 2008; 35:215-21.

16. De Giorgi V, Gori A, Gandini S, et al. Oestrogen receptor beta and melanoma: a comparative study. Br J Dermatol 2013; 168: 513–9
17. 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: 30-6
18. Dika E, Fanti PA, Vaccari S, *et al.* Oestrogen and progesterone receptors in melanoma and nevi: an immunohistochemical study. *Eur J Dermatol* (2017); 27(3): 254-9
19. Bataille V. It's Not All Sunshine: Non-sun-related Melanoma Risk-factors. Acta Derm Venereol. 2020;100(11):adv00137. doi:10.2340/00015555-3492
20. Schwartz MR, Luo L, Berwick M. Sex Differences in Melanoma. Curr Epidemiol Rep. 2019;6(2):112-118. doi:10.1007/s40471-019-00192-7
21. Wei EX, Qureshi AA, Han J, et al. Trends in the diagnosis and clinical features of melanoma in situ (MIS) in US men and women: A prospective, observational study. J Am Acad Dermatol. 2016;75(4):698-705. doi:10.1016/j.jaad.2016.05.011
22. Joosse A, de Vries E, Eckel R, Nijsten T, Eggermont AM, Hölzel D, Coebergh JW, Engel J; Munich Melanoma Group. Gender differences in melanoma survival: female patients have a decreased risk of metastasis. J Invest Dermatol. 2011 Mar;131(3):719-26. doi: 10.1038/jid.2010.354. Epub 2010 Dec 9. PMID: 21150923.
23. Crocetti E, Fancelli L, Manneschi G, et al. Melanoma survival: sex does matter, but we do not know how. Eur J Cancer Prev. 2016;25(5):404-409. doi:10.1097/CEJ.0000000000000190
24. De Vries E, Nijsten TE, Visser O, et al. Superior survival of females among 10,538 Dutch melanoma patients is independent of Breslow thickness, histologic type and tumor site. Ann Oncol. 2008;19(3):583-589. doi:10.1093/annonc/mdm498
25. Joosse A, van der Ploeg AP, Haydu LE, et al. Sex differences in melanoma survival are not related to mitotic rate of the primary tumor. Ann Surg Oncol. 2015;22(5):1598-1603. doi:10.1245/s10434-014-4166-8
26. Joosse A, Collette S, Suciu S, et al. Sex is an independent prognostic indicator for survival and relapse/progression-free survival in metastasized stage III to IV melanoma: a pooled analysis of five European organisation for research and treatment of cancer randomized controlled trials. J Clin Oncol. 2013;31(18):2337-2346. doi:10.1200/JCO.2012.44.5031
27. Behbahani S, Maddukuri S, Cadwell JB, Lambert WC, Schwartz RA. Gender differences in cutaneous melanoma: Demographics, prognostic factors, and survival outcomes [published online ahead of print, 2020 Aug 5]. Dermatol Ther. 2020;e14131. doi:10.1111/dth.14131
28. Holman DM, Ding H, Guy GP Jr, Watson M, Hartman AM, Perna FM. Prevalence of Sun Protection Use and Sunburn and Association of Demographic and Behaviorial Characteristics With Sunburn Among US Adults. JAMA Dermatol. 2018;154(5):561-568. doi:10.1001/jamadermatol.2018.0028

29. Courtenay WH, McCreary DR, Merighi JR. Gender and ethnic differences in health beliefs and behaviors. J Health Psychol. 2002;7(3):219-231. doi:10.1177/1359105302007003216
30. Lukic M, Jareid M, Weiderpass E, Braaten T. Coffee consumption and the risk of malignant melanoma in the Norwegian Women and Cancer (NOWAC) Study. BMC Cancer. 2016;16:562. Published 2016 Jul 29. doi:10.1186/s12885-016-2586-5
31. Liu F, Bessonova L, Taylor TH, Ziogas A, Meyskens FL Jr, Anton-Culver H. A unique gender difference in early onset melanoma implies that in addition to ultraviolet light exposure other causative factors are important. Pigment Cell Melanoma Res. 2013;26(1):128-135. doi:10.1111/pcmr.12035
32. Liu-Smith F, Farhat AM, Arce A, et al. Sex differences in the association of cutaneous melanoma incidence rates and geographic ultraviolet light exposure. J Am Acad Dermatol. 2017;76(3):499-505.e3. doi:10.1016/j.jaad.2016.08.027
33. Nosrati A, Wei ML. Sex disparities in melanoma outcomes: the role of biology. Arch Biochem Biophys 2014; 563:42–50.
34. Balaton BP, Dixon-McDougall T, Peeters SB, Brown CJ. The eXceptional nature of the X chromosome. Hum Mol Genet. 2018;27(R2):R242-R249. doi:10.1093/hmg/ddy148
35. Visconti A, Sanna M, Bataille V, Falchi M. Genetics plays a role in nevi distribution in women. Melanoma Manag. 2020 Mar 17;7(1):MMT35. doi: 10.2217/mmt-2019-0019. PMID: 32399173; PMCID: PMC7212503.
36. Visconti A, Ribero S, Sanna M, Spector TD, Bataille V, Falchi M. Body site-specific genetic effects influence naevus count distribution in women. Pigment Cell Melanoma Res. 2020;33(2):326-333. doi:10.1111/pcmr.12820
37. Schoemaker MJ, Swerdlow AJ, Higgins AJ, Wright AF, Jacobs PA, et al. Mortality in women with Turner syndrome in Great Britain: a national survey. J Clin Endocrinol Metab 2008; 93: 4735–4742.
38. Hernando B, Ibarrola-Villava M, Fernandez LP, et al. Sex-specific genetic effects associated with pigmentation, sensitivity to sunlight, and melanoma in a population of Spanish origin. Biol Sex Differ. 2016;7:17. Published 2016 Mar 18. doi:10.1186/s13293-016-0070-1
39. Gupta S, Artomov M, Goggins W, Daly M, Tsao H. Gender Disparity and Mutation Burden in Metastatic Melanoma. J Natl Cancer Inst. 2015;107(11):djv221. Published 2015 Aug 20. doi:10.1093/jnci/djv221
40. Robbins HA, Clarke CA, Arron ST, et al. Melanoma Risk and Survival among Organ Transplant Recipients. J Invest Dermatol. 2015;135(11):2657-2665. doi:10.1038/jid.2015.312
41. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol. 2016;16(10):626-638. doi:10.1038/nri.2016.90
42. Gubbels Bupp MR, Potluri T, Fink AL, Klein SL. The Confluence of Sex Hormones and Aging on Immunity. Front Immunol. 2018 Jun 4;9:1269.

43. Kim YJ, Kim K, Lee KH, Kim J, Jung W. Immune expression signatures as candidate prognostic biomarkers of age and gender survival differences in cutaneous melanoma. Sci Rep. 2020;10(1):12322. Published 2020 Jul 23. doi:10.1038/s41598-020-69082-z
44. Bouman A, Heineman MJ, Faas MM. Sex hormones and the immune response in humans. Hum Reprod Update. 2005;11(4):411-423. doi:10.1093/humupd/dmi008
45. Giltay EJ, Fonk JC, von Blomberg BM, Drexhage HA, Schalkwijk C, Gooren LJ. In vivo effects of sex steroids on lymphocyte responsiveness and immunoglobulin levels in humans. J Clin Endocrinol Metab. 2000;85(4):1648-1657. doi:10.1210/jcem.85.4.6562
46. Fish EN. The X-files in immunity: sex-based differences predispose immune responses. Nat Rev Immunol. 2008;8(9):737-744. doi:10.1038/nri2394
47. Bynoe MS, Grimaldi CM, Diamond B. Estrogen up-regulates Bcl-2 and blocks tolerance induction of naive B cells. Proc Natl Acad Sci U S A. 2000;97(6):2703-2708. doi:10.1073/pnas.040577497
48. Kanda N, Tamaki K. Estrogen enhances immunoglobulin production by human PBMCs. J Allergy Clin Immunol. 1999;103(2 Pt 1):282-288. doi:10.1016/s0091-6749(99)70503-8
49. Lin PY, Sun L, Thibodeaux SR, et al. B7-H1-dependent sex-related differences in tumor immunity and immunotherapy responses. J Immunol. 2010;185(5):2747-2753. doi:10.4049/jimmunol.1000496
50. Bellenghi M, Puglisi R, Pontecorvi G, De Feo A, Carè A, Mattia G. Sex and Gender Disparities in Melanoma. Cancers (Basel). 2020;12(7):1819. Published 2020 Jul 7. doi:10.3390/cancers12071819
51. Kemeny MM, Busch E, Stewart AK, Menck HR. Superior survival of young women with malignant melanoma. Am J Surg. 1998;175(6):437-445. doi:10.1016/s0002-9610(98)00070-1
52. 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 Nov;47(17):2607-17. doi: 10.1016/j.ejca.2011.04.023. Epub 2011 May 26. PMID: 21620689
53. 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 May 15;173(10):1192-202. doi: 10.1093/aje/kwq503. Epub 2011 Mar 16. PMID: 21415032
54. Olsen CM, Pandeya N, Dusingize JC, et al. Reproductive factors, hormone use and melanoma risk: an Australian prospective cohort study [published online ahead of print, 2020 Aug 27]. Br J Dermatol. 2020;10.1111/bjd.19498. doi:10.1111/bjd.19498
55. Donley GM, Liu WT, Pfeiffer RM, McDonald EC, Peters KO, Tucker MA, Cahoon EK. Reproductive factors, exogenous hormone use and incidence of melanoma among women in the United States. Br J Cancer. 2019 Apr;120(7):754-760. doi: 10.1038/s41416-019-0411-z. Epub 2019 Feb 28. PMID: 30814688; PMCID: PMC6461881.

56. Støer NC, Botteri E, Ghiasvand R, Busund M, Vangen S, Lund E, Veierød MB, Weiderpass E. Reproductive factors and risk of melanoma: a population-based cohort study. Br J Dermatol. 2019 Aug;181(2):282-289. doi: 10.1111/bjd.17771. Epub 2019 May 13. PMID: 30748007.
57. Lea CS, Holly EA, Hartge P, et al. Reproductive risk factors for cutaneous melanoma in women: a case-control study. Am J Epidemiol. 2007;165(5):505-513. doi:10.1093/aje/kwk040
58. Karagas MR, Zens MS, Stukel TA, et al. Pregnancy history and incidence of melanoma in women: a pooled analysis. Cancer Causes Control. 2006;17(1):11-19. doi:10.1007/s10552-005-0281-y
59. Kaae J, Andersen A, Boyd HA, Wohlfahrt J, Melbye M. Reproductive history and cutaneous malignant melanoma: a comparison between women and men. Am J Epidemiol. 2007;165(11):1265-1270. doi:10.1093/aje/kwm015
60. Li Z, Gu M, Cen Y. Age at first birth and melanoma risk: a meta-analysis. Int J Clin Exp Med. 2014;7(12):5201-5209. Published 2014 Dec 15.
61. Gandini S, Lazzeroni M, Peccatori FA, et al. The risk of extra-ovarian malignancies among women with endometriosis: A systematic literature review and meta-analysis. Crit Rev Oncol Hematol. 2019;134:72-81. doi:10.1016/j.critrevonc.2018.12.009
62. Kvaskoff M, Mesrine S, Fournier A, Boutron-Ruault MC, Clavel-Chapelon F. Personal history of endometriosis and risk of cutaneous melanoma in a large prospective cohort of French women. Arch Intern Med. 2007;167(19):2061-2065. doi:10.1001/archinte.167.19.2061
63. Farland LV, Lorrain S, Missmer SA, Dartois L, Cervenka I, Savoye I, Mesrine S, Boutron-Ruault MC, Kvaskoff M. Endometriosis and the risk of skin cancer: a prospective cohort study. Cancer Causes Control. 2017 Oct;28(10):1011-1019. doi: 10.1007/s10552-017-0939-2. Epub 2017 Aug 10. PMID: 28799019; PMCID: PMC5693730
64. Salvini C, Scarfì F, Fabroni C, Taviti F. Melanoma and pregnancy. G Ital Dermatol Venereol. 2017;152(3):274-285. doi:10.23736/S0392-0488.17.05558-4
65. Motosko CC, Bieber AK, Pomeranz MK, Stein JA, Martires KJ. Physiologic changes of pregnancy: A review of the literature. Int J Womens Dermatol. 2017;3(4):219-224. Published 2017 Oct 21. doi:10.1016/j.ijwd.2017.09.003
66. Panicker VV, Riyaz N, Balachandran PK. A clinical study of cutaneous changes in pregnancy. J Epidemiol Glob Health. 2017;7(1):63-70. doi:10.1016/j.jegh.2016.10.002
67. Foucar E, Bentley TJ, Laube DW, Rosai J. A histopathologic evaluation of nevocellular nevi in pregnancy. Arch Dermatol. 1985;121(3):350-354.
68. Bieber AK, Martires KJ, Driscoll MS, Grant-Kels JM, Pomeranz MK, Stein JA. Nevi and pregnancy. J Am Acad Dermatol. 2016;75(4):661-666. doi:10.1016/j.jaad.2016.01.060
69. Chan MP, Chan MM, Tahan SR. Melanocytic nevi in pregnancy: histologic features and Ki-67 proliferation index. J Cutan Pathol. 2010;37(8):843-851. doi:10.1111/j.1600-0560.2009.01491.x

70. Aktürk AS, Bilen N, Bayrämgürler D, Demirsoy EO, Erdogan S, Kiran R. Dermoscopy is a suitable method for the observation of the pregnancy-related changes in melanocytic nevi. J Eur Acad Dermatol Venereol. 2007;21(8):1086-1090. doi:10.1111/j.1468-3083.2007.02204.x
71. Van Rooij N, Adams A, De'Ambrosis B, Nathan V, Hayward N, Whiteman D. Cluster of pregnancy-associated melanoma: A case report and brief update. J Dermatol. 2020;47(9):1054-1057. doi:10.1111/1346-8138.15455
72. Driscoll MS, Stein AJ, Grant-Kels JM. Melanoma and pregnancy. In: UpToDate, Post, TW (Ed), UpToDate, Waltham, MA,2019.
73. Daryanani D, Plukker JT, De Hullu JA, Kuiper H, Nap RE, Hoekstra HJ. Pregnancy and early-stage melanoma. Cancer. 2003;97(9):2248-2253. doi:10.1002/cncr.11321
74. Lens MB, Rosdahl I, Ahlbom A, et al. Effect of pregnancy on survival in women with cutaneous malignant melanoma. J Clin Oncol. 2004;22(21):4369-4375. doi:10.1200/JCO.2004.02.096
75. Jones MS, Lee J, Stern SL, Faries MB. Is Pregnancy-Associated Melanoma Associated with Adverse Outcomes? J Am Coll Surg. 2017 Jul;225(1):149-158. doi: 10.1016/j.jamcollsurg.2017.02.011. Epub 2017 Apr 27. PMID: 28457692; PMCID: PMC5484731.
76. O'Meara AT, Cress R, Xing G, Danielsen B, Smith LH. Malignant melanoma in pregnancy. A population-based evaluation. Cancer. 2005;103(6):1217-1226. doi:10.1002/cncr.20925
77. Johansson AL, Andersson TM, Plym A, Ullenhag GJ, Møller H, Lambe M. Mortality in women with pregnancy-associated malignant melanoma. J Am Acad Dermatol. 2014;71(6):1093-1101. doi:10.1016/j.jaad.2014.09.018
78. Ribero S, Longo C, Dika E, et al. Pregnancy and melanoma: a European-wide survey to assess current management and a critical literature overview. J Eur Acad Dermatol Venereol. 2017;31(1):65-69. doi:10.1111/jdv.13722
79. Tellez A, Rueda S, Conic RZ, et al. Risk factors and outcomes of cutaneous melanoma in women less than 50 years of age. J Am Acad Dermatol. 2016;74(4):731-738. doi:10.1016/j.jaad.2015.11.014
80. Andersson TM, Johansson AL, Fredriksson I, Lambe M. Cancer during pregnancy and the postpartum period: A population-based study. Cancer. 2015;121(12):2072-2077. doi:10.1002/cncr.29325
81. Stensheim H, Møller B, van Dijk T, Fosså SD. Cause-specific survival for women diagnosed with cancer during pregnancy or lactation: a registry-based cohort study. J Clin Oncol. 2009;27(1):45-51. doi:10.1200/JCO.2008.17.4110
82. Møller H, Purushotham A, Linklater KM, Garmo H, Holmberg L, Lambe M, Yallop D, Devereux S. Recent childbirth is an adverse prognostic factor in breast cancer and melanoma, but not in Hodgkin lymphoma. Eur J Cancer. 2013 Nov;49(17):3686-93. doi: 10.1016/j.ejca.2013.06.047. Epub 2013 Aug 6. PMID: 23932699.

83. Filoni A, Mariano M, Cameli N. Melasma: How hormones can modulate skin pigmentation. J Cosmet Dermatol. 2019;18(2):458-463. doi:10.1111/jocd.12877
84. Verdier-Sevrain S, Yaar M, Cantatore J, Traish A, Gilchrest BA. Estradiol induces proliferation of keratinocytes via a receptor mediated mechanism. FASEB J. 2004;18(11):1252-1254. doi:10.1096/fj.03-1088fje
85. Cervenka I, Mahamat-Saleh Y, Savoye I, et al. Oral contraceptive use and cutaneous melanoma risk: a French prospective cohort study. Int J Cancer. 2018;143(10):2390-2399. doi:10.1002/ijc.31644
86. Cahoon EK, Kitahara CM, Ntowe E, et al. Female Estrogen-Related Factors and Incidence of Basal Cell Carcinoma in a Nationwide US Cohort. J Clin Oncol. 2015;33(34):4058-4065. doi:10.1200/JCO.2015.62.0625
87. Colucci R, Buzzoni C, Dragoni F, et al. Drug consumption and melanocytic nevi: correlation between oral contraceptives or hormone replacement therapy and a high melanocytic nevi count. Eur J Cancer Prev. 2020;29(4):338-341. doi:10.1097/CEJ.0000000000000545.
88. Pfahlberg A, Hassan K, Wille L, Lausen B, Gefeller O. Systematic review of case-control studies: oral contraceptives show no effect on melanoma risk. Public Health Rev. 1997;25(3-4):309-315.
89. Karagas MR, Stukel TA, Dykes J, et al. A pooled analysis of 10 case-control studies of melanoma and oral contraceptive use. Br J Cancer. 2002;86(7):1085-1092. doi:10.1038/sj.bjc.6600196
90. 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(19):1469-1475. doi:10.1093/jnci/djr333
91. Cervenka I, Al Rahmoun M, Mahamat-Saleh Y, et al. Exogenous hormone use and cutaneous melanoma risk in women: The European Prospective Investigation into Cancer and Nutrition. Int J Cancer. 2020;146(12):3267-3280. doi:10.1002/ijc.32674
92. De Giorgi V, Gori A, Savarese I, et al. Role of BMI and hormone therapy in melanoma risk: a case-control study. J Cancer Res Clin Oncol. 2017;143(7):1191-1197. doi:10.1007/s00432-017-2387-5.
93. Rebar RW. Social and ethical implications of fertility preservation. Fertil Steril. 2016;105(6):1449-1451. doi:10.1016/j.fertnstert.2016.02.002
94. Joo BS, Park SH, An BM, Kim KS, Moon SE, Moon HS. Serum estradiol levels during controlled ovarian hyperstimulation influence the pregnancy outcome of in vitro fertilization in a concentration-dependent manner. Fertil Steril. 2010;93(2):442-446. doi:10.1016/j.fertnstert.2009.02.066

95. Del Pup L, Peccatori FA, Levi-Setti PE, Codacci-Pisanelli G, Patrizio P. Risk of cancer after assisted reproduction: a review of the available evidences and guidance to fertility counselors. Eur Rev Med Pharmacol Sci. 2018;22(22):8042-8059. doi:10.26355/eurrev_201811_16434
96. Luke B, Brown MB, Spector LG, et al. Cancer in women after assisted reproductive technology. Fertil Steril. 2015;104(5):1218-1226. doi:10.1016/j.fertnstert.2015.07.1135
97. Althuis MD, Scoccia B, Lamb EJ, et al. Melanoma, thyroid, cervical, and colon cancer risk after use of fertility drugs. Am J Obstet Gynecol. 2005;193(3 Pt 1):668-674.
98. Hannibal CG, Jensen A, Sharif H, Kjaer SK. Malignant melanoma risk after exposure to fertility drugs: results from a large Danish cohort study. Cancer Causes Control. 2008; 19(7):759-765.
99. Berk-Krauss J, Bieber AK, Criscito MC, et al. Melanoma risk after in vitro fertilization: A review of the literature. J Am Acad Dermatol. 2018;79(6):1133-1140.e3. doi:10.1016/j.jaad.2018.07.022 Dec;79(6):1133-1140.e3. doi: 10.1016/j.jaad.2018.07.022. Epub 2018 Jul 25. PMID:30055204.
100. Brinton LA, Moghissi KS, Scoccia B, et al. Effects of fertility drugs on cancers other than breast and gynecologic malignancies. Fertil Steril. 2015;104(4):980-988.
101. Calderon-Margalit R, Friedlander Y, Yanetz R, et al. Cancer risk after exposure to treatments for ovulation induction. Am J Epidemiol. 2009;169(3):365-375.
102. Spaan M, van den Belt-Dusebout AW, Schaapveld M, Mooij TM, Burger CW, van Leeuwen FE. Melanoma risk after ovarian stimulation for in vitro fertilization. Hum Reprod. 2015;30(5):1216-1228.
103. Silva Idos S, Wark PA, McCormack VA, et al. Ovulation stimulation drugs and cancer risks: a long-term follow-up of a British cohort. Br J Cancer. 2009;100(11):1824-1831.
104. Kroener L, Dumesic D, Al-Safi Z. Use of fertility medications and cancer risk: a review and update. Curr Opin Obstet Gynecol. 2017 Aug;29(4):195-201. doi: 10.1097/GCO.0000000000000370. PMID: 28538003; PMCID: PMC5551049.
105. Huber C, Bouchardy C, Schaffar R, et al. Antiestrogen therapy for breast cancer modifies the risk of subsequent cutaneous melanoma. Cancer Prev Res (Phila). 2012;5(1):82-88. doi:10.1158/1940-6207.CAPR-11-0332
106. Ricceri F, Fasanelli F, Giraudo MT, et al. Risk of second primary malignancies in women with breast cancer: Results from the European prospective investigation into cancer and nutrition (EPIC). Int J Cancer. 2015;137(4):940-948. doi:10.1002/ijc.29462
107. Santen RJ, Santner SJ, Harvey HA, et al. Marked heterogeneity of aromatase activity in human malignant melanoma tissue. Eur J Cancer Clin Oncol. 1988;24(12):1811-1816. doi:10.1016/0277-5379(88)90090-9
108. Dika E, Patrizi A, Lambertini M, et al. Estrogen Receptors and Melanoma: A Review. Cells. 2019;8(11):1463. Published 2019 Nov 19. doi:10.3390/cells8111463
109. Thornton MJ. The biological actions of estrogens on skin. Exp Dermatol 2002;11:487–502.

110. Hewitt SC, Korach KS. Estrogen receptors: structure, mechanisms and function. Rev Endocr Metab Disord. 2002;3(3):193-200. doi:10.1023/a:1020068224909
111. Kumar R, Zakharov MN, Khan SH, et al. The dynamic structure of the estrogen receptor. J Amino Acids. 2011;2011:812540. doi:10.4061/2011/812540
112. Gu Y, Chen T, López E, et al. The therapeutic target of estrogen receptor-alpha36 in estrogen-dependent tumors. J Transl Med. 2014;12:16. Published 2014 Jan 21. doi:10.1186/1479-5876-12-16
113. Vrtačnik P, Ostanek B, Mencej-Bedrač S, Marc J. The many faces of estrogen signaling. Biochem Med (Zagreb). 2014;24(3):329-342. Published 2014 Oct 15. doi:10.11613/BM.2014.035
114. Le Dily F, Beato M. Signaling by Steroid Hormones in the 3D Nuclear Space. Int J Mol Sci. 2018;19(2):306. Published 2018 Jan 23. doi:10.3390/ijms19020306
115. Marino M, Galluzzo P, Ascenzi P. Estrogen signaling multiple pathways to impact gene transcription. Curr Genomics. 2006;7(8):497-508. doi:10.2174/138920206779315737
116. O'Lone R, Frith MC, Karlsson EK, Hansen U. Genomic targets of nuclear estrogen receptors. Mol Endocrinol. 2004;18(8):1859-1875. doi:10.1210/me.2003-0044
117. Piu F, Aronheim A, Katz S, Karin M. AP-1 repressor protein JDP-2: inhibition of UV-mediated apoptosis through p53 down-regulation. Mol Cell Biol. 2001;21(9):3012-3024. doi:10.1128/MCB.21.9.3012-3024.2001
118. Dos Santos EG, Dieudonne MN, Pecquery R, Le Moal V, Giudicelli Y, Lacasa D. Rapid nongenomic E2 effects on p42/p44 MAPK, activator protein-1, and cAMP response element binding protein in rat white adipocytes. Endocrinology. 2002;143(3):930-940. doi:10.1210/endo.143.3.8678
119. Eyster KM. The Estrogen Receptors: An Overview from Different Perspectives. Methods Mol Biol. 2016;1366:1-10. doi:10.1007/978-1-4939-3127-9_1
120. Barone I, Brusco L, Fuqua SA. Estrogen receptor mutations and changes in downstream gene expression and signaling. Clin Cancer Res. 2010;16(10):2702-2708. doi:10.1158/1078-0432.CCR-09-1753
121. Filardo EJ, Thomas P. Minireview: G protein-coupled estrogen receptor-1, GPER-1: its mechanism of action and role in female reproductive cancer, renal and vascular physiology. Endocrinology. 2012;153(7):2953-2962. doi:10.1210/en.2012-1061
122. Lee HJ, Wall B, Chen S. G-protein-coupled receptors and melanoma. Pigment Cell Melanoma Res. 2008;21(4):415-428. doi:10.1111/j.1755-148X.2008.00478.x
123. Sun M, Xie HF, Tang Y, et al. G protein-coupled estrogen receptor enhances melanogenesis via cAMP-protein kinase (PKA) by upregulating microphthalmia-related transcription factor-tyrosinase in melanoma. J Steroid Biochem Mol Biol. 2017;165(Pt B):236-246. doi:10.1016/j.jsbmb.2016.06.012

124. Ribeiro MPC, Santos AE, Custódio JBA. The activation of the G protein-coupled estrogen receptor (GPER) inhibits the proliferation of mouse melanoma K1735-M2 cells. Chem Biol Interact. 2017;277:176-184. doi:10.1016/j.cbi.2017.09.017
125. Fábián M, Rencz F, Krenács T, et al. Expression of G protein-coupled oestrogen receptor in melanoma and in pregnancy-associated melanoma. J Eur Acad Dermatol Venereol. 2017;31(9):1453-1461. doi:10.1111/jdv.14304
126. Zhou JH, Kim KB, Myers JN, Fox PS, Ning J, Bassett RL, Hasanein H, Prieto VG. Immunohistochemical expression of hormone receptors in melanoma of pregnant women, nonpregnant women, and men. Am J Dermatopathol. 2014 Jan;36(1):74-9. doi: 10.1097/DAD.0b013e3182914c64. PMID: 23812018; PMCID: PMC3795893.
127. Glatthaar H, Katto J, Vogt T, Mahlknecht U. Estrogen Receptor Alpha (ESR1) Single-Nucleotide Polymorphisms (SNPs) Affect Malignant Melanoma Susceptibility and Disease Course. Genet Epigenet. 2016;8:1-6. Published 2016 Feb 24. doi:10.4137/GEG.S31264
128. Marzagalli M, Montagnani Marelli M, 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. Published 2016 Oct 26. doi:10.3389/fendo.2016.00140
129. de Giorgi V, Mavilia C, Massi D, et al. The role of estrogens in melanoma and skin cancer. Carcinogenesis. 2009;30(4):720. doi:10.1093/carcin/bgp025
130. 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(1):30-36. doi:10.1001/archdermatol.2008.537
131. Taylor AH, Al-Azzawi F. Immunolocalisation of oestrogen receptor beta in human tissues. J Mol Endocrinol. 2000;24(1):145-155. doi:10.1677/jme.0.0240145
132. Panasiti V, Barone M, Coppola R, Devirgiliis V, Roberti V, Persichetti P, Carotti S, Morini S. Expression of estrogen receptors in spitz and reed nevi. G Ital Dermatol Venereol. 2019 Jul 26. doi: 10.23736/S0392-0488.19.06376-4. Epub ahead of print. PMID: 31355620
133. Folkerd EJ, Dowsett M. Influence of sex hormones on cancer progression. J Clin Oncol. 2010;28(26):4038-4044. doi:10.1200/JCO.2009.27.4290
134. Fox EM, Davis RJ, Shupnik MA. ERbeta in breast cancer--onlooker, passive player, or active protector?. Steroids. 2008;73(11):1039-1051. doi:10.1016/j.steroids.2008.04.006
135. Hartman J, Ström A, Gustafsson JA. Estrogen receptor beta in breast cancer--diagnostic and therapeutic implications. Steroids. 2009;74(8):635-641. doi:10.1016/j.steroids.2009.02.005
136. Roger P, Sahla ME, Mäkelä S, Gustafsson JA, Baldet P, Rochefort H. Decreased expression of estrogen receptor beta protein in proliferative preinvasive mammary tumors. Cancer Res. 2001;61(6):2537-2541.

137. Hartman J, Edvardsson K, Lindberg K, et al. Tumor repressive functions of estrogen receptor beta in SW480 colon cancer cells. Cancer Res. 2009;69(15):6100-6106. doi:10.1158/0008-5472.CAN-09-0506
138. Drummond AE, Fuller PJ. The importance of ERbeta signalling in the ovary. J Endocrinol. 2010;205(1):15-23. doi:10.1677/JOE-09-0379
139. Thomas CG, Strom A, Lindberg K, Gustafsson JA. Estrogen receptor beta decreases survival of p53-defective cancer cells after DNA damage by impairing G_2/M checkpoint signaling. Breast Cancer Res Treat. 2011;127(2):417-427. doi:10.1007/s10549-010-1011-z
140. Lindberg K, Ström A, Lock JG, Gustafsson JA, Haldosén LA, Helguero LA. Expression of estrogen receptor beta increases integrin alpha1 and integrin beta1 levels and enhances adhesion of breast cancer cells. J Cell Physiol. 2010;222(1):156-167. doi:10.1002/jcp.21932
141. Elder DE, Massi D, Scolyer RA, Willemze R, editors. World Health Organization classification of skin tumors. Lyon: IARC Press; 2018: 48-50.
142. Amin MB, Edge SB, Greene FL, et al., editors. AJCC cancer staging manual. 8th ed. New York: Springer; 2017.
143. College of American Pathologists (2020, October 1st). Protocol for the Examination of Excision Specimens From Patients With Melanoma of the Skin. Version: 4.2.0.0. CAP; 2020. https://www.cap.org/protocols-and-guidelines/cancer-reporting-tools/cancer-protocol-templates
144. Dika E, Lambertini M, Patrizi A, et al. Frequency of somatic mutations in head and neck melanoma: A single-institution experience. Pigment Cell Melanoma Res. 2020;33(3):515-517. doi:10.1111/pcmr.12864
145. Morgese F, Sampaolesi C, Torniai M, et al. Gender Differences and Outcomes in Melanoma Patients. Oncol Ther. 2020;8(1):103-114. doi:10.1007/s40487-020-00109-1
146. Olsen CM, Thompson JF, Pandeya N, Whiteman DC. Evaluation of Sex-Specific Incidence of Melanoma [published correction appears in JAMA Dermatol. 2020 May 1;156(5):604]. JAMA Dermatol. 2020;156(5):553-560. doi:10.1001/jamadermatol.2020.0470
147. Whiteman DC, Stickley M, Watt P, Hughes MC, Davis MB, Green AC. Anatomic site, sun exposure, and risk of cutaneous melanoma. J Clin Oncol. 2006;24(19):3172-3177. doi:10.1200/JCO.2006.06.1325
148. Whiteman DC, Watt P, Purdie DM, Hughes MC, Hayward NK, Green AC. Melanocytic nevi, solar keratoses, and divergent pathways to cutaneous melanoma. J Natl Cancer Inst. 2003;95(11):806-812. doi:10.1093/jnci/95.11.806
149. De Giorgi V, Scarfì F, Gori A, et al. Nevi and Breslow thickness in melanoma: sex differences?. Melanoma Res. 2020;30(2):179-184. doi:10.1097/CMR.0000000000000579
150. Mitchell DL, Fernandez AA, Garcia R, et al. Acute exposure to ultraviolet-B radiation modulates sex steroid hormones and receptor expression in the skin and may contribute to the sex bias of

melanoma in a fish model. Pigment Cell Melanoma Res. 2014;27(3):408-417. doi:10.1111/pcmr.12213

151. Borrás C, Gambini J, López-Grueso R, Pallardó FV, Viña J. Direct antioxidant and protective effect of estradiol on isolated mitochondria. Biochim Biophys Acta. 2010;1802(1):205-211. doi:10.1016/j.bbadis.2009.09.007

152. Kanda N, Watanabe S. 17beta-estradiol, progesterone, and dihydrotestosterone suppress the growth of human melanoma by inhibiting interleukin-8 production. J Invest Dermatol. 2001;117(2):274-283. doi:10.1046/j.1523-1747.2001.01422.x

153. Dessinioti C, Dimou N, Geller AC, et al. Distinct Clinicopathological and Prognostic Features of Thin Nodular Primary Melanomas: An International Study from 17 Centers. J Natl Cancer Inst. 2019;111(12):1314-1322. doi:10.1093/jnci/djz034

154. Abramova N, Hubbard J, Schertz J, Richter E. Safety of Follitropin Alfa/Lutropin Alfa for Stimulation of Follicular Development. Drug Saf. 2019;42(3):453-461. doi:10.1007/s40264-018-0742-3

155. Sinnamon AJ, Sharon CE, Song Y, et al. The prognostic significance of tumor-infiltrating lymphocytes for primary melanoma varies by sex. J Am Acad Dermatol. 2018;79(2):245-251. doi:10.1016/j.jaad.2018.02.066

156. Zhang D, Xu X, Ye Q. Metabolism and immunity in breast cancer [published online ahead of print, 2020 Oct 19]. Front Med. 2020;10.1007/s11684-020-0793-6. doi:10.1007/s11684-020-0793-6

157. Mitkov M, Joseph R, Copland J 3rd. Steroid hormone influence on melanomagenesis. Mol Cell Endocrinol. 2015;417:94-102. doi:10.1016/j.mce.2015.09.020

158. Cuzick J, Sestak I, Forbes JF, et al. Use of anastrozole for breast cancer prevention (IBIS-II): long-term results of a randomised controlled trial [published correction appears in Lancet. 2020 Feb 15;395(10223):496]. Lancet. 2020;395(10218):117-122. doi:10.1016/S0140-6736(19)32955-1

159. Andersson S, Sundberg M, Pristovsek N, et al. Insufficient antibody validation challenges oestrogen receptor beta research [published correction appears in Nat Commun. 2017 Nov 29;8:16164]. Nat Commun. 2017;8:15840. Published 2017 Jun 15. doi:10.1038/ncomms15840

160. Auriemma M, Di Nicola M, Varrati S, et al. Mole modifications following controlled ovarian stimulation for assisted reproduction technologies. J Eur Acad Dermatol Venereol. 2015;29(10):1913-1917. doi:10.1111/jdv.13065

161. Liu N, Liu Z, Liu X, et al. Identification of an Immune-Related Prognostic Signature Associated With Immune Infiltration in Melanoma. Front Genet. 2020;11:1002. Published 2020 Aug 28. doi:10.3389/fgene.2020.01002

162. Lens MB, Reiman T, Husain AF. Use of tamoxifen in the treatment of malignant melanoma. *Cancer*. 2003;98(7):1355-1361. doi:10.1002/cncr.11644

163. Beguerie JR, Xingzhong J, Valdez RP. Tamoxifen vs. non-tamoxifen treatment for advanced melanoma: a meta-analysis. *Int J Dermatol*. 2010;49(10):1194-1202. doi:10.1111/j.1365-4632.2010.04529.x

164. Chen P, Sheikh S, Ahmad A, Ali SM, Ahmad MU, Ahmad I. Orally administered endoxifen inhibits tumor growth in melanoma-bearing mice. *Cell Mol Biol Lett*. 2018;23:3. Published 2018 Jan 3. doi:10.1186/s11658-017-0068-7

165. Zhao L, Huang S, Mei S, et al. Pharmacological activation of estrogen receptor beta augments innate immunity to suppress cancer metastasis. *Proc Natl Acad Sci U S A*. 2018;115(16):E3673-E3681. doi:10.1073/pnas.1803291115

166. Natale CA, Li J, Zhang J, et al. Activation of G protein-coupled estrogen receptor signaling inhibits melanoma and improves response to immune checkpoint blockade. *Elife*. 2018;7:e31770. Published 2018 Jan 16. doi:10.7554/eLife.31770

167. Baffa R, Fassan M, Volinia S, et al. MicroRNA expression profiling of human metastatic cancers identifies cancer gene targets. *J Pathol*. 2009;219(2):214-221. doi:10.1002/path.2586

168. Maillot G, Lacroix-Triki M, Pierredon S, et al. Widespread estrogen-dependent repression of micrornas involved in breast tumor cell growth. *Cancer Res*. 2009;69(21):8332-8340. doi:10.1158/0008-5472.CAN-09-2206

169. Adams BD, Furneaux H, White BA. The micro-ribonucleic acid (miRNA) miR-206 targets the human estrogen receptor-alpha (ERalpha) and represses ERalpha messenger RNA and protein expression in breast cancer cell lines. *Mol Endocrinol*. 2007;21(5):1132-1147. doi:10.1210/me.2007-0022

170. Pandey DP, Picard D. miR-22 inhibits estrogen signaling by directly targeting the estrogen receptor alpha mRNA [published correction appears in Mol Cell Biol. 2009 Sep;29(17):4873]. *Mol Cell Biol*. 2009;29(13):3783-3790. doi:10.1128/MCB.01875-08

171. Zhao Y, Deng C, Lu W, et al. let-7 microRNAs induce tamoxifen sensitivity by downregulation of estrogen receptor α signaling in breast cancer. *Mol Med*. 2011;17(11-12):1233-1241. doi:10.2119/molmed.2010.00225

172. Milevskiy MJG, Gujral U, Del Lama Marques C, et al. MicroRNA-196a is regulated by ER and is a prognostic biomarker in ER+ breast cancer. *Br J Cancer*. 2019;120(6):621-632. doi:10.1038/s41416-019-0395-8

173. Xiao B, Zhang W, Chen L, et al. Analysis of the miRNA-mRNA-lncRNA network in human estrogen receptor-positive and estrogen receptor-negative breast cancer based on TCGA data. *Gene*. 2018;658:28-35. doi:10.1016/j.gene.2018.03.011

174. Vrtačnik P, Ostanek B, Mencej-Bedrač S, Marc J. The many faces of estrogen signaling. *Biochem Med (Zagreb)*. 2014;24(3):329-342. Published 2014 Oct 15. doi:10.11613/BM.2014.035

175. Nagpal N, Sharma S, Maji S, et al. Essential role of MED1 in the transcriptional regulation of ER-dependent oncogenic miRNAs in breast cancer. *Sci Rep*. 2018;8(1):11805. Published 2018 Aug 7. doi:10.1038/s41598-018-29546-9

176. Vidal-Gómez X, Pérez-Cremades D, Mompeón A, Dantas AP, Novella S, Hermenegildo C. MicroRNA as Crucial Regulators of Gene Expression in Estradiol-Treated Human Endothelial Cells. *Cell Physiol Biochem*. 2018;45(5):1878-1892. doi:10.1159/000487910

177. Felicetti F, De Feo A, Coscia C, et al. Exosome-mediated transfer of miR-222 is sufficient to increase tumor malignancy in melanoma. *J Transl Med*. 2016;14:56. Published 2016 Feb 24. doi:10.1186/s12967-016-0811-2

178. Godshalk SE, Paranjape T, Nallur S, et al. A Variant in a MicroRNA complementary site in the 3' UTR of the KIT oncogene increases risk of acral melanoma. *Oncogene*. 2011;30(13):1542-1550. doi:10.1038/onc.2010.536