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Sex-related Differences in Toxicities caused by Treatment of Advanced Melanoma Patients

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Desidero esprimere la mia più sincera gratitudine a tutte le persone che hanno reso possibile la realizzazione di questa tesi.

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Contents

Abstract

- **Background**: Immunotherapy and target therapy have revolutionized melanoma treatment, however adverse events (AEs) are still frequent and might impact treatment adherence. Female sex was demonstrated to be associated with a higher risk of AEs from cytotoxic therapy related to cancer treatment. This study aims to conduct a systematic review and meta-analysis to investigate Sex effect on adverse events.

- **Methods**: We extracted data from independent studies published until April 2024 with information regarding toxicity by S/G. Only studies that involved patients with melanoma as primary disease were included. Summary Odds ratios OR (sOR) estimates were obtained through random-effects models, and 95% Confidence Intervals (CI) were estimated. I^2 was used to evaluate the between-study heterogeneity. Publication bias was investigated with Begg and Egger tests.

- **Results**: Information regarding toxicity and sex were extracted from seventy articles. A significant increase in AE related to the thyroid was observed in women (sOR=2.00, 95%CI[1.41-2.85], $\mathbf{I^2}$=29). S/G did not affect Grade III-IV, Dermatological, Gastrointestinal, Hypophysis, Kidney, Liver or Ocular toxicities. The increased risk of AE related to the thyroid was mainly seen for those undergoing first-line treatments, (sOR=2.17, 95%CI[1.42-3.32], I^2=44), and in both those who underwent mono and combo therapy. Women

who underwent combo-immunotherapy showed an increased risk of developing dermatological AE, as well. Women undergoing TT (target therapy) had a significant increased risk of dermatological (sOR= 1.99, 95%CI[1.55-2.55], I^2=64) AE and grade III-IV AE (sOR = 1.6, 95%CI[1.08-2.38], I^2=64).

- **Conclusion**: This study supports and extends the discussion on the impact of S/G on the development of toxicities. Women have an increased risk of AE related to the thyroid, mainly seen during first-line treatment, and showed an increased risk of dermatological and severe AE when treated with TT. Women should be monitored closely for precursor signs of thyroid-related AE, as well as dermatological and generally severe AE, to preserve a good treatment adherence.

Chapter 1

Introduction

1.1 Rationale of the Study

Understanding the subtle changes in hormone regulation and adaptive immune responses between the sexes is crucial for the effectiveness of customized treatment approaches. These variations can, in fact, have a substantial impact on how the disease progresses, how susceptible it is to different diseases, and how the body responds to therapy. Different sex hormones, such estrogen and testosterone, have different impacts on the immune system. For instance, testosterone can modify Th2 responses while tending to reduce Th1 lymphocyte-mediated immune responses. Depending on the patient's sex, this varied modulation may lead to therapies being more or less successful.

These factors make it vital to comprehend whether a patient's sex affects how beneficial a particular treatment type may be for advanced melanoma, especially considering the range of medicines currently available. In instance, there are several therapeutic options for advanced melanoma, such as radiation therapy, immunotherapy, targeted treatments, and surgery. Understanding how different treatments interact with a patient's immune system and metabolism, whether they are male or female, is essential to maximizing the effectiveness of that treatment.

Several studies have demonstrated how patients' health has been im-

pacted by various treatment techniques based on their sex . For instance, studies on the effectiveness of immunotherapy have revealed differences in response between males and females, which have been partially ascribed to variances in sex hormone levels and innate and adaptive immune responses. In order to improve therapy outcomes, it may be necessary to further research sex variations in the effects of immune checkpoint inhibitor treatments, such as PD-1 and CTLA-4 inhibitors.

A meta-analysis of patient studies with advanced melanoma that highlights health data broken down by therapy type and sex may be very helpful in understanding these dynamics. Such an investigation might lead to more accurate and individualized medicine by revealing important distinctions about the best kind of treatment for each sex.

Health care providers can get valuable knowledge from this analysis to customize treatment plans and improve results for patients of both sexes. For instance, it might turn out that men might gain more from certain strategies, but women would respond better to particular combinations of medicines. Personalized medicine will become a viable and successful reality by improving patients' quality of life and survival rates by accounting for these variances.

1.2 Objectives and Hypotheses

The goal of the current meta-analysis was to assess the toxicity of cancer therapies in terms of enhancing patients' quality of life and clinical results in those with advanced melanoma. The apparent consistency of the findings from earlier research and the necessity for a thorough quantitative synthesis that can offer more accurate treatment recommendations are the driving forces behind interest in this topic. Given the consistency of the research, it appears likely that female patients with advanced melanoma may be more likely to experience different side effects.

1.2.1 Specific aims of the meta-analysis

The main objective is to conduct a meta-analysis to determine whether there is an overall difference in side effects for immunotherapy and target therapy by the sex of patients. The pursuit of this goal has also resulted in a focus on analyzing individual toxicities in addition to generic ones, restricting the research field.

1.2.2 Hypotheses to be tested

Based on multiple research, the primary hypothesis to be investigated is whether the toxicity of oncological therapy varies by sex in patients with advanced melanoma. More specifically, prior data suggests that therapies will cause a greater number of adverse events in female patients.

1.3 Significance of the Study

This research could lead to a deeper understanding of the risks of the occurrence of adverse events caused by the modern therapies for patients with advanced melanoma. Healthcare professionals will be able to adapt the treatment for patients with advanced melanoma depending on whether they are male or female, in order to induce a better quality of life during treatment.

1.4 Structure of the Thesis

The thesis is formatted in accordance with a scientific article's format.

The study context will need some space, which we shall provide in Chapter 2. An overview of advanced melanoma is provided in this scope, together with information on risk factors, prognostic aspects, diagnostic, epidemiological, and therapeutic options, as well as adverse events (AEs). It also reveals variations in immune responses and hormone regulation between the sexes, which may impact how well the current therapy works.

The procedures needed to conduct the analyses and, consequently, the different processes needed to put together a meta-analysis are explained in Chapter 3.

The data gathered from the completed research are presented in the results portion of the thesis, which is located in Chapter 4. In actuality, tables and graphs are used to facilitate the presentation of all results in a logical order.

This is followed by Chapter 5 on the discussion. That section is the part where the results are argued by giving answers to the "whys" set forth in the introduction. New hypotheses and arguments are made based on contradictions between the data obtained and those found in other scientific articles.

Finally, it closes with the last chapter, which is the section that summarizes the conclusions drawn from the work presented and offers further analyses that can be carried out in the future.

Chapter 2

Background

2.1 Advanced Melanoma

Even though it's thought of as a modern illness, melanoma has been known since antiquity. Hippocrates documented the first incidence in the fifth century B.C., and evidence of it has been discovered in pre-Columbian mummies dating back roughly 2,400 years. The first documented examples in Western medical literature date from 1651 and 1757. John Hunter removed a melanoma surgically for the first time in 1787 after mistaking it for a fungus growth. William Norris noted in 1820 that melanomas were caused by moles and postulated a genetic basis for the illness. In order to avoid recurrence, he was also the first to advocate for broad excision margins. Herbert Snow proposed the excision of lymph nodes in 1892 as a way to stop cancer from spreading. For almost a century, the primary methods of treating melanoma were surgical excision and lymph node removal; metastases were not an option. The advancement in melanoma treatment did not occur until the 1940s, when chemotherapies were introduced [1]. The prognosis for advanced melanoma, which is classified as an incurable or metastatic illness, has generally been bleak. The development of immune checkpoint inhibitors and targeted treatments has significantly changed the therapy landscape for melanoma throughout the last ten years. Even with these develop-

ments, there are still issues to deal with, such as managing brain metastases, uncommon melanoma subtypes such uveal and mucosal melanoma, and the rise in resistance to existing treatments [2].

2.1.1 Incidence

A malignant tumor of melanocytes, or cells that produce melanin (pigment) in the basal layer of the epidermis, is called melanoma. Due to their neural crest ancestry, melanocytes express a wide range of chemicals and signaling components that facilitate migration and metastasis during malignant transformation. Melanoma is responsible for about 80% of skin cancer mortality, even though it only makes up 1% of skin cancers. There are numerous clinical subtypes of melanoma that vary in molecular profile, demography, and presentation. The most prevalent type of cutaneous melanomas, especially in fair-skinned people, is superficial spreading melanoma (SSM), which has a low Breslow thickness and often a good prognosis depending on when it is diagnosed. Darker-skinned ethnic groups are more prone to experience acral lentiginous melanoma, which develops from the glabrous skin of the nail beds, soles of the feet, and palms of the hands. Melanoma can also develop from mucosal or uveal tissue, albeit this is less common and most likely unrelated to UV exposure. The prognosis for uveal melanoma is especially bad, with over 50% of patients progressing to stage IV illness [3, 4].

Global Incidence Rates

Outside the United States, melanoma incidence is quite high. With melanoma incidence rates around double that of the US, Australia has the highest incidence of the disease. Since 1982, the Australian Institute of Health and Welfare has compiled data on the incidence of melanoma in Australia. These data are accessible till 2007. 10,342 cases of melanomas were reported in 2007; the incidence, age-standardized to the Australian population of 2001, was 46.7 per 100,000. By the age of 75, Australians

have a 1 in 28 chance of getting melanoma. From 1982 to 2007, Australia's average yearly change in skin melanoma incidence rates was 2.3%, which is similar to changes in the US. The incidence has not increased to the same extent in Europe as it has in Australia and the United States. In the UK, the incidence for males and women between 1998 and 2002 ranged from 6.9 to 10.7 per 100,000 and 7.1 to 12.6 per 100,000, respectively. Germany, Norway, and Switzerland had the highest incidence rates for males, with age-standardized rates of 12.7, 14.2, and 18.6 per 100,000, respectively. The highest age-standardized rates for females are found in Denmark, Norway, Iceland, and Switzerland, at 14.1, 14.6, 19, and 19.6 per 100,000, respectively. White people's incidence rates of melanoma often decline with distance from the equator. In contrast, the opposite pattern—with a larger prevalence in the North—is seen in Western Europe. This is thought to be the case because people from northern Europe with lighter skin tone migrate to southern Europe, where they are exposed to intense and sporadic sun [4, 5].

Trends Over Time

Recent changes in lifestyle, including greater intermittent exposure to ultraviolet (UV) radiation, tanning, travel to sunny regions, and use of sunbeds, are thought to be a major contributing factor to the rise in melanoma incidence. It's also thought that increased awareness, surveillance, and early discovery played a role in the rise in incidence. The majority of those with light pigmentation features who are of European heritage have been affected by the rise in skin cancer incidence. It is also possible that a rise in the number of melanomas diagnosed has resulted, at least partially, from increased dermatologic surveillance of melanoma patients and increasing awareness of skin cancer [6].

Age and Sex Distribution

- **Age**: Melanoma is becoming more and more prevalent all over the world and peaks in the seventh and eighth decades of life. Numerous high-risk communities, including those in Australia, New Zealand, and northern Europe, exhibit this trend. However, in the US, the incidence peaks in the sixth decade of life, with 44.9 percent of melanomas detected in Americans between the ages of 55 and 74. Melanoma is one of the most prevalent cancers in adolescents and young adults, while having the lowest incidence among those under 40. It is the second most frequent malignancy among women in the United States between the ages of 20 and 29. In a similar vein, melanoma is one of the malignancies that young adults get diagnosed with most frequently worldwide.

- **Sex**: Men and women are affected by melanoma in different ways, which reflects variations in occurrence between populations. Women are more likely than men to get melanoma in their teens and early adult years, most likely as a result of the extensive indoor tanning practices used by women. Men are more likely to have melanoma after the age of 40. Males are often more prone to melanoma, which may be impacted by androgens. In the US, the yearly incidence is 17.3 cases per 100,000 cases for women and 29.2 cases per 100,000 cases for men. All ethnic groups have this elevated rate among men; non-Hispanic Caucasian males have greater rates, while African American and Asian males have lower rates. The one exception is the American Indian/Alaska Native population, where women are significantly more likely than men to have this incidence. In the past, communities with low prevalence in higher latitudes, like those in Scotland and Canada, have reported significantly higher rates of melanoma in women. According to reports, women in Scotland are twice as likely as males to develop

melanoma. In contrast, most populations in low-to-mid latitudes, including those in the United States, Australia, and New Zealand, have a higher prevalence of melanoma in males than in women. Overall, women's advantage in high-latitude, low-incidence populations has been neutralized by the rise in male melanoma incidence; even in these places, men now outweigh women [4, 7] [2.1, 2.2].

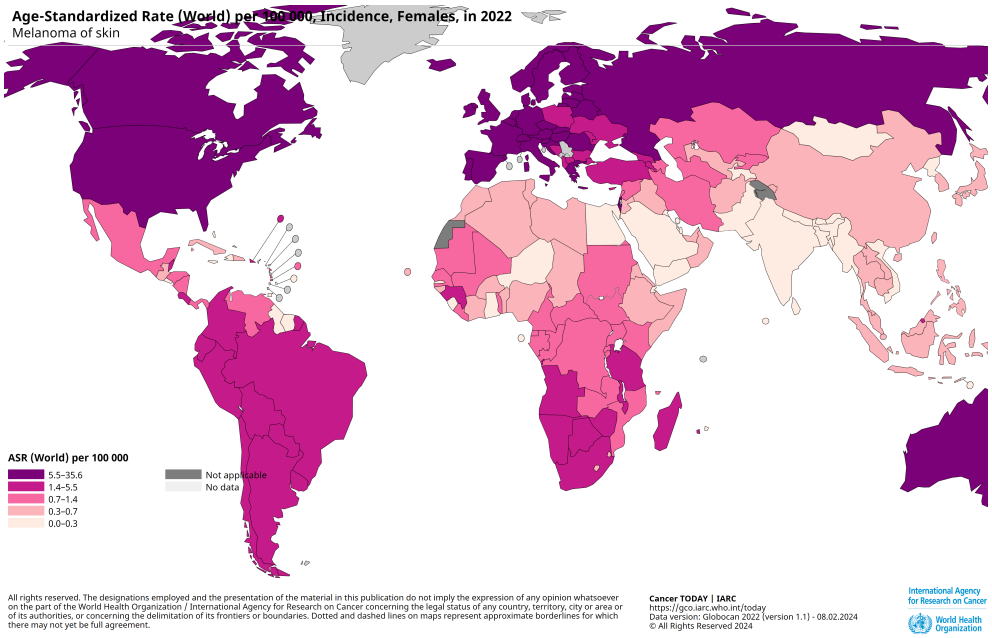

Figure 2.1: *Age standardized (World) incidence rates, Melanoma of skin, females, all ages*

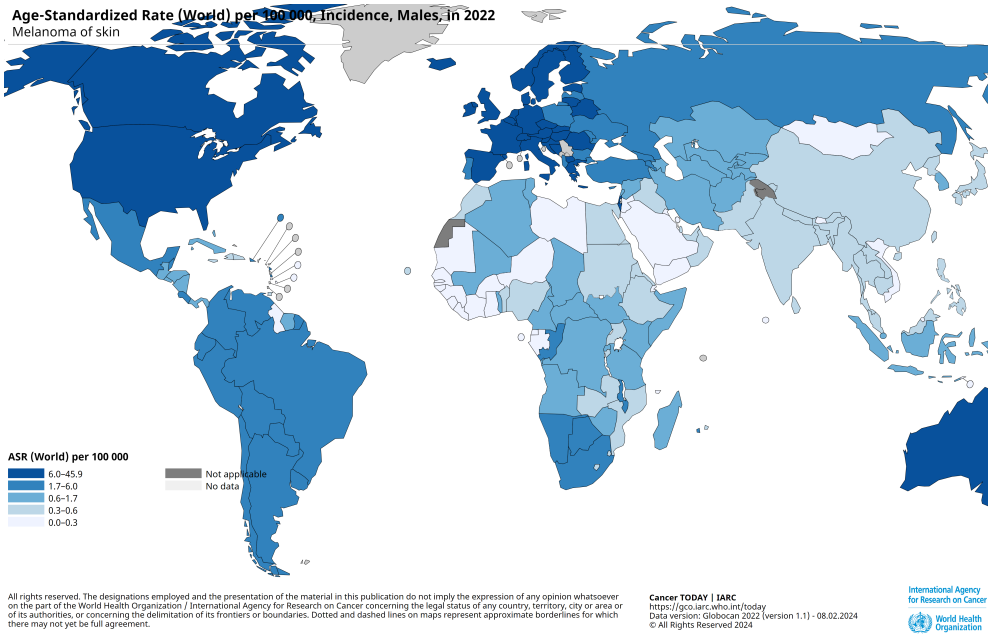

Figure 2.2: *Age standardized (World) incidence rates, Melanoma of skin, males, all ages*

Geographic and Environmental Influences

Global comparisons of melanoma incidence are possible thanks to national statistics, although significant differences in incidence within nations with diverse populations—like the US, New Zealand, Australia, Israel, and South Africa—can be obscured. Furthermore, when a nation spans multiple degrees of latitude, like Australia does, changes in melanoma incidence within that nation may go unnoticed. Melanoma incidence is lower in those with darker skin types and higher in Caucasian residents with lighter skin, across all countries. Variations in altitude may also affect the incidence of melanoma. There is a correlation between a higher incidence of melanoma in high altitude regions and low latitude locations in countries. Comparably, people who routinely participate in high-altitude sports like mountaineering have noticeably greater incidence rates. UV irradiation is correlated with altitude; however, height can also cause changes in ozone absorption, decreased cloud cover, and enhanced surface reflection from snow cover [4, 7].

In conclusion, a mix of lifestyle, environmental, and genetic factors affect the incidence of metastatic melanoma. Comprehending these variables is crucial in formulating efficacious preventive measures and public health protocols intended to mitigate the global incidence of melanoma.

2.1.2 Diagnosis

Due to its simplicity and convenience of use, the current clinicopathologic classification of melanoma is widely used in clinical practice. The four primary clinicopathologic subtypes of this categorization are nodular melanoma, lentigo maligna, acral lentiginous melanomas, and superficial spreading melanoma. Histopathological results, a subjective field that can have issues with reliability across observers, are a major component of the classification. Subtyping becomes arbitrary since many melanomas show overlapping histopathologic patterns within the same lesion, even when some of them clearly meet the clinical and pathologic criteria of a particular subtype. Acral lentiginous melanomas, for instance, exhibit a wide range of histopathologic characteristics, including those seen in the other three categories. Moreover, extrinsic prognostic criteria like Breslow depth and ulceration are added to the pathology report to guide clinical therapy, rather than the categorization itself incorporating prognostic information. All melanomas are classified based on a linear model of progression, wherein lesions that occur in situ are thought to be early obligatory lesions that develop into invasive lesions and then spread to other locations. All melanoma subtypes are treated similarly, mostly on the basis of melanoma thickness, even if the development of melanomas might differ greatly. The World Health Organization has recently unveiled a new melanoma classification that combines clinicopathologic criteria with epidemiologic and genetic data. There are now nine subcategories in this categorization, including mucosal and extremely uncommon melanomas.

- **Clinical Diagnosis**: The ABCD mnemonic was first used in the

1980s to help in melanoma diagnosis. It was recently expanded to ABCDE by adding the "E" criteria for "evolution," which increased diagnostic sensitivity. These requirements include recent evolution, asymmetry, uneven boundaries, color variability, and a diameter of at least 6 mm. Using these parameters, the estimated diagnostic accuracy is 65%. Because dermoscopy uses polarized light and 10x magnification to detect subcutaneous tissues, it has greatly improved the identification of melanocytic neoplasms. Atypical pigmented networks, blue-white veils, irregular striae, and atypical vascular patterns are among the characteristics that are highly correlated with melanoma. The sensitivity and specificity of several diagnostic methods, including Menzies' approach, CASH criteria, and 7-point checklist, are improved for diagnosing melanoma. Despite dermoscopy's success, a 2022 Cochrane review [8] found that there was insufficient evidence to support its effectiveness, especially when it came to lowering biopsy rates, cutting expenses, and enhancing clinical outcomes. The Pigmented Lesion Assay (PLA), a test that examines stratum corneum RNA to find genetic markers linked to melanoma, is another recent invention. Although the test's sensitivity of 91% and high negative predictive value have been demonstrated, research is currently being done on how to use it in real-world scenarios.

In summary, whereas existing techniques can diagnose melanoma more accurately, more research is required to confirm and refine these strategies for use in clinical settings.

- **Histopathological Diagnosis**: The gold standard for diagnosing melanoma remains histological, even with the development of advanced molecular diagnostics. However, there is a high rate of disagreement among pathologists due to the intrinsic subjectivity of the histologic diagnosis of melanocytic neoplasms. Typically, thick, bulky melanomas don't cause diagnostic issues; but, biopsies

of progressively thinner and smaller tumors have brought attention to the interobserver reliability issue. Only 25% of Spitz and atypical nevi and 45% of atypical Spitz tumor, severely atypical nevi, and melanoma in situ showed concordance in a concordance analysis of melanocytic neoplasms. These percentages are deemed insufficient for a reliable diagnostic tool. This low concordance rate suggests that a wide range of melanocytic neoplasms are treated with varying criteria, which has a major effect on therapeutic therapy. As a result, pathologists are using molecular supplementary diagnostic techniques that are more accurate. Traditionally, immunohistochemical staining has been primarily used to confirm that the neoplasm is melanocytic, and its diagnostic utility has been restricted. But as our knowledge of the molecular markers of melanomas has grown, a number of immunohistochemical stains—like p16 and PRAME—that play more important diagnostic roles have become accessible. In numerous gene expression profiling tests for uveal melanoma prognosis, melanoma diagnosis, and biopsy decision guidance, PRAME is overexpressed in melanomas and other cancers. The sensitivity range for PRAME IHC in the diagnosis of melanoma is 67% to 94%. Despite having a reduced sensitivity of 20% to 35%, S100 and SOX10 are essential in the detection of desmoplastic fusiform cell melanomas. Additional helpful IHC stains for diagnosing melanoma include p16, whose expression decline is highly associated with the diagnosis of melanoma. While it's not always helpful to distinguish between Spitz nevus and spitzoid melanoma, staining for BRAF, BAP1, and cKit status has become popular to help direct targeted therapy [9].

2.1.3 Prognosis

For melanoma staging, medical professionals have historically depended on the American Joint Committee on Cancer (AJCC) recommendations.

The seventh edition of the AJCC melanoma staging system was superseded by the eighth edition, which went into effect in 2018. The definition of the T1a and T1b stages is one of the primary modifications. The cutoff for these stages in the seventh edition was $\leq$ 1 mm Breslow depth. The eighth edition reduced this limit to less than 0.8 mm. Although the full impact of this adjustment is still unclear, it has encouraged more patients to consider sentinel biopsy. According to population-based research, the new smaller depth threshold had no discernible effect on the sentinel lymph node positive rate. On paraffin-embedded sections, qRT-PCR technology has also made it possible to design a number of prognostic assays based on gene expression profiling. The 31-gene DecisionDx-Melanoma assay from Castle Biosciences was created in the US to offer predictive risk stratification separate from the AJCC staging system. When predicting the likelihood of metastasis or recurrence in patients with stage I, II, or III melanoma, this test is especially helpful. It has been demonstrated that the test results can independently predict the likelihood of metastatic spread, which can lead to more individualized patient care. These developments point to a shift in melanoma treatment toward personalized medicine, where clinical, pathological, and molecular features are all taken into consideration to steer patient care and therapy [9].

2.1.4 Risk factor

These days, melanoma is thought to be a complex illness brought on by a combination of environmental exposure and genetic predisposition. There are other factors that raise the possibility of metastatic melanoma. To identify people who might benefit from tailored prevention programs and early detection efforts, it is imperative to comprehend these risk factors.

- **UV Radiation Exposure**: Because UV radiation has a genotoxic effect, it is the most significant and theoretically modifiable envi-

ronmental risk factor for the development of malignant melanoma. Elwood et al.'s studies have demonstrated a link between sun exposure and melanoma, leading them to conclude that sporadic solar exposure is a significant factor in determining melanoma risk [10]. Sunburns are linked to an increased chance of getting melanoma, especially if they happened as a youngster. On the other hand, actinic keratosis and nonmelanomatous skin malignancies are more commonly linked to prolonged and persistent sun exposure. Melanoma can also develop as a result of artificial UV exposure, such as that from tanning beds. Actually, compared to sun exposure from regular outdoor activities, the amount of UVA absorbed during a typical tanning booth session is substantially higher. The psoralen-UV-A radiation photochemotherapy (PUVA) used to treat psoriasis has also been linked to a higher risk of melanoma, which highlights the significance of UV exposure monitoring and limitation. These results emphasize the significance of primary prevention in lowering the risk of malignant melanoma by limiting exposure to UV radiation, both artificial and natural. The prevention of melanoma necessitates public awareness and educational efforts about sun protection, clothing to wear, and avoiding sunburn, especially in children [11].

- **Genetic Factors**: One of the most potent risk factors for melanoma is a family history, which suggests inherited causes. The genetic foundation of melanoma has been clarified by studies during the last 20 years. In families with hereditary melanoma, Tsao et al. showed an autosomal dominant inheritance pattern, with common mutations in the CDKN2A gene (p16) and uncommon mutations in the CDK4 gene. Individuals who are genetically predisposed to melanoma experience a better prognosis, many primary melanomas, precursor lesions such dysplastic nevi, and superficially invasive tumors [12]. They also acquire melanoma before the age of

40. Melanoma risk is increased by family cancer syndromes, including Lynch syndrome type II, Li-Fraumeni syndrome, and familial retinoblastoma [11].

- **Presence of Moles**: In the context of melanoma, the number of melanocytic nevi, family history, and genetic predisposition are the most significant host risk factors. Benign collections of melanocytes or nevus cells, known as melanocytic nevi, can be acquired or congenital. In about 25% of instances, a preexisting nevus develops simultaneously with melanoma. Furthermore, there exists a positive correlation between the total number, size, and type of nevi and the risk of melanoma. Patients with over 100 nevi are seven times more likely to develop melanoma, according to a recent meta-analysis. Nevi that are larger than 5 mm and giant nevi that are larger than 20 cm are linked to a notably increased risk of melanoma. An unusual nevus typically has a flat component, varies in coloration, has an uneven, asymmetric contour, and has vague borders. Its size is usually at least 5 mm. In situations of nonfamilial melanoma, dysplastic nevus predisposes to arise in between 29 and 49 percent of cases. Atypical nevi are a strong predictor of elevated risk; the risk of melanoma is enhanced by even a single nevus with atypical features, and the risk increases sixfold in the presence of five atypical nevi. In younger individuals, melanomas that arise in the context of prior nevi typically occur locally on the trunk and are frequently of the superficial spreading form. The gradual radial growth of this kind of melanoma may eventually give way to a more aggressive vertical growth phase. Careful observation and, in certain situations, genetic testing to find genes that may predispose to the development of melanoma are necessary for the clinical management of individuals with multiple nevi, atypical nevi, or a family history of melanoma [11, 13].

- **Phenotype Characteristics**: Melanoma risk is around 50% higher in those with phenotypic features such as red hair, fair complexion, lots of freckles, light-colored eyes, sun sensitivity, and difficulty to tan. These genetic characteristics are linked to decreased UV protection, which increases the risk of skin damage and melanoma in those who carry them [11].

Reducing the burden of metastatic melanoma requires identifying and addressing these risk factors through sun protection measures, routine skin exams, and genetic counseling for individuals who are genetically predisposed to the condition.

2.2 Treatment

Thanks to developments in cancer cell biology and immunology, melanoma, which was formerly thought to be among the most difficult malignancies to treat with conventional therapeutic methods like chemotherapy and radiation, has significantly improved in recent years. These developments have completely changed the way metastatic melanoma is treated, resulting in remarkable responses to novel immunotherapies and targeted medicines that significantly increase patient survival and quality of life. Chemotherapeutic drugs have been the primary therapeutic option for treating metastatic melanoma for a long time. Benefits to survival were, however, scarce. Promising tactics in recent years have included CTLA-4 blocking to enhance anticancer immune responses and blocking signal transduction pathways like BRAF inhibition. Furthermore, a number of novel approaches have surfaced, such as kinase inhibitors, vaccinations, and immunotherapy. Patients with BRAF V600E mutations have benefited greatly with BRAF inhibition, with survival and quality of life markedly improved. Immune checkpoint drugs, such ipilimumab (anti-CTLA-4) and pembrolizumab (anti-PD-1) antibodies, have produced remarkable outcomes. Some patients have experienced

long-lasting responses, and their overall survival has significantly improved. The emphasis has recently switched to therapeutic combinations, which seek to optimize treatment success by combining the advantages of several medicines. These developments provide patients with metastatic melanoma fresh hope and substantial benefits as they constitute a breakthrough in the treatment of this illness [14, 15].

2.2.1 Immunotherapy

Immunotherapy has shown promise in improving survival in people with incurable illness and lowering the chance of melanoma recurrence following surgical resection. Many melanoma patients will still experience disease progression or recurrence in spite of the tremendous advancements in melanoma treatment.

Anti-CTLA-4

Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) is crucial for immune tolerance and is implicated in antitumor immune responses. CTLA-4 is a cell surface receptor, homolog of CD28, expressed on both regulatory and conventional T cells, and is upregulated upon T cell activation. Binding of B7 to CTLA-4 initiates an inhibitory signaling cascade, suppressing T-cell function. Anti-CTLA-4 monoclonal antibodies prevent CD28 from binding to B7, thereby increasing T-cell activation and enhancing immune recognition of tumor cells [16]. A monoclonal antibody called imilimumab prevents CTLA-4 from doing its job. In a trial, ipilimumab (3 mg/kg) was administered to patients with metastatic melanoma in addition to the gp100 vaccination, or in addition to the vaccine alone. The results showed clinical efficacy in treating melanoma. When compared to patients who received the gp100 vaccine alone, patients treated with ipilimumab had a better overall survival rate: the median overall survival for ipilimumab plus gp100 was 10.0 months, 10.1 months for ipilimumab alone, and 6.4 months for gp100 alone. Ten to

fifteen percent of patients experienced grade ≥ 3 adverse events, with fatigue, dermatitis, and diarrhea being the most frequent toxicities. These findings led to the FDA's 2011 approval of ipilimumab (3 mg/kg) for the treatment of inoperable stage IV melanoma [17]. Next, using a 10 mg/kg dosage of ipilimumab, dacarbazine with ipilimumab was compared with dacarbazine alone in patients with metastatic melanoma that had not previously received treatment. Compared to dacarbazine alone, imilimumab with dacarbazine showed a noticeably prolonged overall survival period (11.2 vs. 9.1 months). In contrast, the group treated with dacarbazine alone experienced a considerably lower rate of grade ≥ 3 adverse events (56.3% vs. 27.5%) than the group treated with ipilimumab and dacarbazine [18]. Ipilimumab doses of 3 mg/kg and 10 mg/kg were found to be effective, thus they were compared in a phase 3 study involving patients who had metastatic or incurable melanoma. In comparison to 3 mg/kg, the 10 mg/kg dose demonstrated better overall survival (median 15.7 months vs. 11.5 months) and relapse-free survival. On the other hand, the 10 mg/kg group saw a greater incidence of grade ≥ 3 adverse events (34.0% vs. 18.2%)[19]. Following evidence of clinical effectiveness in the metastatic context, ipilimumab was investigated as an adjuvant treatment. When compared to a placebo, ipilimumab 10 mg/kg increased overall survival for stage III melanoma that had been fully excised. However, for high-risk resected melanoma, ipilimumab 3 and 10 mg/kg were compared with high-dose interferon alpha-2b (HDI) due to the significant incidence of major side events that were found. When compared to HDI, ipilimumab 3 mg/kg demonstrated better overall survival. The toxicity of ipilimumab 10 mg/kg was much higher than that of HDI, but trends in favor of the drug did not achieve statistical significance. 2015 saw the FDA approve ipilimumab at a dose of 10 mg/kg as an adjuvant treatment for fully resected stage III melanoma; however, more current research suggests using a dose of 3 mg/kg. Another anti-CTLA4 antibody that has been considered for metastatic melanoma treatment is tremelimumab.

Tremelimumab, however, did not show a survival advantage over dacarbazine or regular temozolomide in one research. Tremelimumab is not being utilized in clinical practice for the treatment of melanoma, which may have affected these results due to the usage of ipilimumab [20].

Anti-PD-1

While its ligand, programmed death protein-1 receptor (PD-L1), is extensively distributed on both immune and nonimmune cells, the receptor itself is found on the surface of lymphocytes. T-cell activity is decreased with PD-1 and PD-L1 binding, which enhances immunological tolerance. Many types of cancer, including melanoma, have overexpressed PD-L1, which helps cancer cells avoid the immune system. Immune identification of malignant cells and their subsequent immune-mediated death are restored when PD-1 is inhibited [21].

- **Pembrolizumab**: Using pembrolizumab and nivolumab, two PD-1-specific monoclonal antibodies, the anticancer effect of PD-1 inhibition has been shown. Pembrolizumab showed potential clinical efficacy for treating advanced melanoma in the phase 1 KEYNOTE-001 trial when it was used in advanced solid tumors. Later, pembrolizumab showed improvement in progression-free survival in the KEYNOTE-002 trial compared with conventional treatment after progression over ipilimumab. Compared to chemotherapy, pembrolizumab caused fewer grade ≥ 3 adverse events. Pembrolizumab became the new standard of therapy as a result of these outcomes. Pembrolizumab was compared to ipilimumab in the KEYNOTE-006 study for the treatment of advanced melanoma, showing better overall survival with reduced toxicity and progression-free survival. The FDA approved pembrolizumab as the first-line treatment for metastatic or incurable melanoma [22–24]. Pembrolizumab has proven to be effective when used as an adjuvant for stage III melanoma at high risk. The KEYNOTE-054 trial demonstrated a signif-

icantly longer recurrence-free survival when pembrolizumab was compared with placebo. Adverse events happened at a pace akin to what was seen in the Checkmate 238 study with nivolumab [25, 26]. Later, in the KEYNOTE-716 study, pembrolizumab was investigated as adjuvant therapy for high-risk stage II melanoma, demonstrating a noteworthy decrease in death or recurrence. In 2021, the FDA approved pembrolizumab as an adjuvant treatment for melanoma in stages IIB or IIC following full resection [27]. Neoadjuvant immunotherapy is becoming more popular as a treatment option for diseases that can be treated. Neoadjuvant pembrolizumab significantly improved event-free survival in the SWOG S1801 study as compared to standard adjuvant therapy. Lenvatinib, an anti-angiogenic/multiple RTK inhibitor, and pembrolizumab together represent a promising combination, as demonstrated by the NeoPeLe study, which demonstrated partial pathologic response in a notable percentage of patients with resectable stage III melanoma. The outcomes of these investigations provide credence to the possible application of neoadjuvant immunotherapy for treatable melanoma [20, 28, 29].

- **Nivolumab**: A human IgG4 monoclonal antibody that targets PD-1 is called nivolumab. A phase 1 experiment conducted in 2012 showed that Nivolumab was effective in treating individuals with advanced solid malignancies, such as melanoma. The FDA expedited approval in 2014 for the treatment of metastatic melanoma as a result of these findings. When compared to chemotherapy for advanced melanoma, nivolumab had better objective response rates (27% vs. 10%) and sustained responses (32 vs. 13 months) in the CheckMate 037 trial. Compared to chemotherapy, nivolumab had a grade ≥ 3 adverse event rate of 14%, indicating higher tolerance [30, 31]. In patients with advanced melanoma that had not had prior treatment, nivolumab significantly improved overall

and progression-free survival as compared to dacarbazine in the CheckMate 066 trial. These long-term gains were validated by a 5-year follow-up [32]. Additionally approved as adjuvant therapy was nivolumab. In patients with resected stage IIIB, IIIC, or IV melanoma, nivolumab had better relapse-free survival when compared to ipilimumab in the CheckMate 238 trial. Compared to ipilimumab, which had a grade ≥ 3 adverse event rate of 45.9%, nivolumab had a more favorable side effect profile, with a rate of 14.4% [20, 33–36].

- **Ipilimumab e Nivolumab**: In treating metastatic melanoma, ipilimumab and nivolumab together demonstrated synergistic benefits. The highest tolerated doses of nivolumab (1 mg/kg) and ipilimumab (3 mg/kg) were found in a phase I investigation. A phase II trial that contrasted the combination with ipilimumab alone later showed improvements in progression-free survival and the objective response rate (61% vs. 11%). The FDA approved the combination for advanced melanoma [37, 38]. The combination was found to be more effective than single therapies in the phase III CheckMate-067 research, which demonstrated a median overall survival of 72.1 months with the combination, 36.9 months with nivolumab, and 19.9 months with ipilimumab. When using the combination, grade ≥ 3 adverse events were more common (59%) than when using the individual therapies [39–41]. For patients with BRAF mutations, one study demonstrated a survival benefit by starting with nivolumab/ipilimumab compared with dabrafenib/-trametinib. A phase II study indicated that adding tocilizumab to the combination could reduce toxicity without compromising efficacy. In a subsequent scenario, the IMMUNED study shown that the combination improved the free survival of recidiva compared to the placebo, but not significantly compared to nivolumab alone. Phase III CheckMate 915 study has not demonstrated a significant

improvement in recidiva survival when combined with nivolumab alone in resectable melanoma [20, 42–44].

2.2.2 Target Therapy

New treatments like targeted therapy have significantly reduced mortality rates despite a continuous rise in melanoma prevalence worldwide [4, 45]. Specifically for BRAF V600E/K mutation-positive melanoma, targeted therapy mostly involve inhibitors of the MAPK signaling system, such as BRAF and MEK inhibitors [46–48]. Many patients with metastatic melanoma have shown improved overall survival (OS) and objective response rate (ORR) after using these medications, which have proven to be highly effective. Drug resistance is still a problem, though, particularly after extended use [49, 50].

BRAF and MEK inhibitors

The RAF, MEK, and ERK kinases are involved in MAPK signaling pathways. A BRAF mutation, which hyperactivates the MAPK signaling pathway and impairs melanocyte survival, differentiation, and proliferation, is present in around 50% of patients with metastatic melanoma (more than 90% of these patients have BRAF V600E mutations) [51, 52]. Inhibitors of BRAF (vemurafenib, dabrafenib, and encorafenib) or MEK (cobimetinib, trametinib, and binimetinib) can impede this carcinogenic signaling.

- **Vemurafenib and Cobimetinib**: The first oral inhibitor licensed in 2011 for this kind of melanoma was vemurafenib, which was followed by the powerful MEK inhibitor cobimetinib. The BRIM 7 trial demonstrated a median progression-free survival (PFS) of 13.7 months and validated an objective response rate (ORR) of 87% when vemurafenib and cobimetinib were taken together. With a considerable improvement in median overall survival (22.5 months

compared to 17.4 months with vemurafenib alone) and improving survival rates over time, the global CoBRIM study provided more evidence of the combination's efficacy [53, 54]. Tumor burden and serum lactate dehydrogenase (LDH) levels are examples of prognostic factors that have been found to have an impact on treatment outcome. Individuals with modest tumor burden and normal LDH had superior long-term results. On the other hand, the significant level of toxicity observed in the group receiving cobimetinib and vemurafenib indicated the necessity for methods to control side effects [54, 55]. To further enhance outcomes for patients with BRAF V600-positive melanoma, a novel approach combines BRAF/MEK combination therapy with an immune checkpoint inhibitor, atezolizumab. This combination is being investigated in the IMspire150 trial, particularly in individuals who had significant baseline LDH. According to preliminary data, PFS significantly improves, particularly in subgroups with low PD-L1 expression [56, 57]. To sum up, combination therapy combining atezolizumab and BRAF/MEK inhibitors may be a game-changer for the treatment of metastatic melanoma, including those with brain metastases, by providing a new avenue for improving therapeutic efficacy and tolerance [58].

- **Dabrafenib and Trametinib**: An oral MEK inhibitor is trametinib; an oral BRAF inhibitor is dabrafenib. After trametinib was licensed in 2013, dabrafenib was added to treat unresectable metastatic melanoma with the BRAF V600E/V600K mutation. The combination of trametinib and dabrafenib led to enhanced median overall survival (OS) (25.1 months vs. 18.7 months) and progression-free survival (PFS) (11 months vs. 8.8 months with dabrafenib alone), according to the COMBI d study, a randomized, double-blind phase 3 trial. Additionally, the 2-year survival rate for this combination was 51%, as opposed to 42% for the group receiv-

ing dabrafenib alone [59, 60]. The COMBI I trial assessed the effectiveness of dabrafenib, trametinib, and spartalizumab (an anti-PD-1 antibody) in combination with dabrafenib and trametinib versus placebo; nevertheless, the combination demonstrated only modest benefit, with a low rate of complete responses and no discernible improvement in PFS. Furthermore, the effects of dabrafenib and trametinib combined for adjuvant therapy following surgical resection of high-risk melanoma were investigated. Patients with totally resected stage III melanoma with BRAF V600 mutations who received dabrafenib and trametinib treatment demonstrated increased distant metastasis-free survival (DMFS) and a substantial decrease in recurrence rate when compared to adjuvant placebo treatment, according to the COMBI-AD trial [55, 61]. Lastly, treatment for pyrexia (fever) brought on by trametinib and dabrafenib was discussed. The goal of the COMBI-APlus trial was to lower the incidence of severe pyrexia-related adverse events and to improve pyrexia management. The trial's results showed a significant reduction in pyrexia-related adverse events [62, 63]. In conclusion, dabrafenib plus trametinib is currently the accepted course of treatment for stage III melanoma with a BRAF V600E/K mutation when used as an adjuvant. This combination of drugs has been shown to significantly lower the risk of recurrence and control related adverse effects [64].

2.2.3 Combination Therapy

Clinical trials merging immunological and targeted therapies are one exciting field of study that could eliminate the need to choose which treatment to begin with. It has been found that BRAF-MEK inhibitors alter the immunological milieu in tumors by elevating CD8-positive T cells and melanoma-specific antigens like gp100 and MART-1, which may help the immune system recognize tumor cells. Clinical trials are currently inves-

tigating whether combining immunotherapy therapies with BRAF-MEK inhibitors will increase the frequency of long-lasting responses without unacceptably high toxicity [65]. For instance, ipilimumab and dabrafenib were investigated in a phase 1 trial in patients with BRAF-mutated melanoma, both with and without trametinib. However, the triple combination arm of the trial was stopped due to significant side events, including grade 3 colitis worsened by perforation. Dabrafenib and ipilimumab combination arm is still in progress [66]. In a different research, the anti-PD-L1 antibody durvalumab is combined with dabrafenib and trametinib. Tumor biopsies revealed evidence of immune activation following therapy, including a rise in the number of CD8+ T cells that infiltrate tumors. A reported 69% of patients receiving this combination therapy showed persistent responses, with no exacerbation of immunotherapy-related side effects [67]. In order to ascertain whether combining targeted therapies and immunotherapies is beneficial, future research will concentrate on the length of responses and overall survival, even though the response rate of 69% does not necessarily surpass that predicted from BRAF-MEK inhibitor monotherapy.

2.2.4 Surgery

Tumor excision is the primary treatment for melanoma at all stages. The melanoma and some of the surrounding normal tissue are removed via a broad local excision. If the excision leaves a large wound, it could be necessary to cover the surgical site with skin harvested from another area of the body using a skin graft. Techniques like sentinel node biopsy and lymph node mapping are used to assess whether cancer has progressed to the lymph nodes. The first lymph nodes to get discharge from the main tumor site are the sentinel lymph nodes. Blue dye, or radioactive material, is injected close to the tumor and travels to these sentinel lymph nodes via lymphatic channels. A pathologist removes the identified sentinel lymph node surgically and looks for cancer cells under a microscope.

Should cancerous cells be discovered, more lymph nodes might need to be removed through a process known as a lymphadenectomy. A sentinel lymph node can occasionally be seen in many lymph node groups. Some individuals may get chemotherapy as adjuvant therapy following surgical removal of visible melanoma in order to eliminate any remaining cancer cells and lower the chance of cancer recurrence. Surgery may be necessary to treat symptoms and stop the disease from spreading if melanoma has affected the lymph nodes, lungs, gastrointestinal system, bones, or brain. This will enhance the patient's quality of life [68].

2.2.5 Radiation Therapy

The indications for irradiation can be categorized into four main groups based on the therapeutic purpose and the appropriate timing of radiotherapy introduction in the management of melanoma. Surgery may not be the primary therapeutic method in the early stages of treatment when radiation therapy is employed instead. After surgery, it can be used as adjuvant therapy to get rid of any remaining tumor cells and lower the chance of recurrence. It can also be applied as palliative therapy and electively [69].

- **Radiotherapy as primary therapy**: Surgery is typically preferred as a curative option for melanoma, with radiation therapy being used infrequently as the primary treatment. Nonetheless, it might be selected if the patient opposes surgery, has serious comorbidities, or is in poor condition. Radiotherapy is most commonly used to treat lentigo malignant melanoma (LMM), especially when it is localized and extensive on an aged patient's face. Only three local recurrences occurred between 13 and 44 months following treatment with radiation or a combination of irradiation and surgical excision in 107 individuals with LMM, according to studies . For LMM, radiotherapy has been demonstrated to be both effective and curative, despite the development of lung metastases and re-

gional lymph nodes in certain individuals. This is particularly true in cases where surgery would cause severe functional or cosmetic impairment [70, 71]. Radiation therapy is thought to be the most successful treatment for inoperable mucosal melanoma (MM), with local control rates above 70%. These rates could rise even higher with the application of high linear energy transfer (LET) radiation. With the exception of melanomas of the oral cavity, which have a higher likelihood of regional failure, radiation therapy should only target the main tumor area in cases without clinical signs of illness in the lymph nodes (N0) [72–74].

- **Adjuvant radiotherapy**: The risk of recurrence, treatment side effects, and the likelihood of a good recovery in the event of a recurrence all play a role in the choice to utilize postoperative radiation after the main lesion has been removed. After local irradiation, there is little chance of major problems because the target tissues are superficial. Narrow or positive surgical margins, early and/or multiple recurrences, significant satellitosis, desmoplasia or neurotropism, and mucosal melanomas (MM) are among the factors that adversely affect local control following wide excision. When satellites of the tumor are histologically observed, the incidence of local recurrence is 12–14% [75, 76]; in desmoplastic tumors, it can reach up to 48% [77, 78]. In these instances, neurotropism and insufficient surgical margins may be linked to local recurrence, particularly in vital head and neck anatomical areas. Postoperative radiation therapy can dramatically lower the incidence of local recurrence in high-risk clinical scenarios [79]. Retrospective studies on mucosal melanoma indicate that surgical radiation therapy enhances results but has little effect on survival. It is advised to employ a combination strategy following nonradical surgery in order to enhance local control even following the removal of sizable primary tumors, especially those involving perineural invasion and

sinus involvement [72].

- **Elective radiotherapy**: Patients who are not candidates for sentinel lymph node biopsy but are at risk for nodal micrometastases may benefit from elective irradiation. Elective regional radiation was proven to be a safe and effective treatment option for 157 individuals with high-risk cutaneous melanoma of the head and neck with lymph node involvement (stages I or II) in a retrospective series. Fifty-seven patients had distant recurrence of the cancer, and fifteen had recurrence in the neck lymph nodes after a median follow-up of 68 months. However, this specific indication is less important in the sentinel lymph node dissection era [80, 81].

- **Palliative radiotherapy**: Life prolongation is not the primary objective of palliative RT; rather, the major goals are to improve the quality of life and lessen the symptoms of the disease. When surgery is neither possible or beneficial for example, when there are numerous metastases or unresectable tumors—palliative radiation therapy (RT) is recommended. It is possible to irradiate any kind of metastasis, including skin, lymphatic, brain, bone, and visceral metastases. The location and amount of the tumor determine its effectiveness; metastatic cells are more radioresistant than primary tumor cells [82]. In 85% of cases, radiation totally resolves small skin lesions, whereas less than 30% of bigger lesions ($>$ 5 cm) respond [83]. In less than a month, more than 65% of patients having radiation therapy for bone metastases experience pain relief [84]. Adjuvant post-surgical radiation therapy reduces pain and extends the disease-free period in situations of imminent pathologic fracture. If the patient is in appropriate condition and the projected survival period is more than six weeks, bone fracture surgery is recommended. Depending on the metastases in weight-bearing bones $>$ 2–3 cm in diameter or with cortical damage $>$ 50%, radiation or

combination treatment is chosen [84]. When surgery or stereotactic radiation are not an option for a patient with brain metastases, whole brain radiation therapy, or WBRT, is frequently administered [85]. In 60–70% of patients, WBRT combined with corticosteroids can temporarily improve health status and extend life by one or two months [69, 86].

2.3 Adverse Events

When treating metastatic melanoma, adverse occurrences, also known as side effects, are frequently taken into account. The management of metastatic melanoma has been transformed by treatment alternatives like as immunotherapy, targeted therapy, and combination methods; nevertheless, these treatments come with a number of drawbacks. Optimizing patient care and treatment results requires an understanding of and ability to manage these adverse occurrences.

Activating the body's immune system to target cancer cells is how immunotherapy, which includes immune checkpoint inhibitors like pembrolizumab, nivolumab, and ipilimumab, functions. These medications may produce immune-related adverse events (irAEs), even if in certain patients they can result in sustained responses and better survival. Immunotherapy-related adverse events (irAEs) that are frequently reported include skin rash, pruritus, diarrhea, colitis, hepatitis, pneumonitis, and hypo- or hyperthyroidism. Corticosteroids and other immunosuppressive medications can usually be used to address these side effects, but timely action and close observation are necessary to avoid more serious consequences [87].

Drugs used in targeted therapy, such as MEK inhibitors (trametinib, cobimetinib) and BRAF inhibitors (vemurafenib, dabrafenib), are intended to block particular biochemical pathways that contribute to the development and spread of melanoma. In individuals with BRAF-mutant

melanoma, these medications can effectively decrease tumors and relieve symptoms; however, they can also have unfavorable side effects, including skin rash, lethargy, nausea, vomiting, diarrhea, arthralgia, myalgia, and increased liver enzymes. Targeted therapy may potentially cause some individuals to develop secondary skin malignancies, such as keratoacanthoma or squamous cell carcinoma [88]

When compared to single-agent therapies, combination therapies, which include immunotherapy with targeted therapy or other forms of treatment, may be more effective. They might, however, potentially raise the chance of side effects because individuals might have overlapping toxicities from several medications. Immunotherapy and targeted therapy side effects are frequently linked to combination therapy, as are possible additive or synergistic effects on the immune system and other organ systems [87].

When treating metastatic melanoma, patients must manage adverse effects with a multidisciplinary team that includes endocrinologists, gastroenterologists, dermatologists, oncologists, and other experts as needed. The key elements of optimal care are timely intervention with appropriate supportive care measures, close monitoring of patients for early indications and symptoms of adverse events, and patient education regarding probable side effects. Furthermore, current research initiatives to find biomarkers predictive of therapy response and toxicity may make it easier to customize treatment plans for specific patients and reduce the possibility of unfavorable outcomes in the road [87].

2.4 Biological diversity between Males and Females

Males and females differ in terms of immune function, illness susceptibility, and treatment responses because of changes in sex, which also affects how hormones and adaptive immune responses are regulated. Below is a

summary of various variations:

2.4.1 Hormonal Regulation

- **Sex Hormones**: The two main sex hormones in men and women, respectively, are testosterone and estrogen, and they both have a significant impact on the immune system. By encouraging B cell maturation, antibody formation, and T cell activation, estrogen strengthens immunological responses. On the other hand, testosterone possesses immunosuppressive properties that result in decreased inflammation and weakened immunological responses [89, 90].

- **Menstrual Cycle**: Immune system performance is impacted by variations in progesterone and estrogen levels during the menstrual cycle. For instance, progesterone levels rise during the luteal phase and have immunosuppressive effects, but estrogen peaks during the follicular phase and results in increased immunological responses [91].

2.4.2 Adaptive Immune Responses

- **B Cells**: Estrogen increases B cell activity, which raises the maturation of affinities and the generation of antibodies. This could be one reason why autoimmune illnesses are more common in women [92].

- **T Cells**: It has been noted that there are sex differences in T cell subsets and cytokine production, with females often displaying higher cellular immunological responses than males. While testosterone tends to decrease Th1-mediated immune responses, which are involved in combating infections, it boosts Th2-mediated immune responses, which are linked to autoimmune and allergy illnesses [93, 94].

- **Immune Senescence**: Male and female immunological functions are affected differentially by aging-related variations in sex hormone levels. While aging men may gradually see a reduction in testosterone levels, which may impact immunological control, postmenopausal women see a decline in estrogen, which can cause immune senescence and increase vulnerability to infections and autoimmune illnesses [93–95].

Chapter 3

Methods

3.1 Study Selection Criteria

This first phase describes the exacting standards that were applied when choosing which research to include for the meta-analysis. Making ensuring the included studies are pertinent, and appropriate for answering the study issue is the main objective.

3.1.1 Inclusion and exclusion criteria for studies

The following section outlines the precise inclusion and exclusion criteria that were put in place to decide which studies might be included in the meta-analysis:

- **Inclusion Criteria**: These outline the necessary qualities that research must have in order for it to be taken into account. These include participant demographics (e.g., patient cohorts, particular animal models), study designs (e.g., randomized controlled trials, cohort studies, case-control studies), interventions that are being looked into, outcome metrics, and other relevant variables.

- **Exclusion Criteria**: These outline circumstances under which a study would not be considered appropriate for inclusion, including insufficient methodological rigor, insufficient primary data, insuffi-

cient follow-up time, or other constraints that would jeopardize the validity and dependability of results.

3.1.2 Types of study-designs

The different study approaches included in the meta-analysis can be categorized, with different study kinds being distinguished from one another:

- **Experimental studies**: Researchers that conduct experiments introduce an intervention and examine its effects. Typically, randomized experimental investigations randomly assign people to groups. Including randomized controlled trials (RCTs), which are intended to assess the effectiveness of particular therapies or interventions.
- **Observational Studies**: Studies that don't attempt to alter who is or is not exposed to a risk factor, diagnostic test, treatment, or other intervention are known as observational studies. Including case-control studies, cohort studies, and other observational designs that shed light on relationships and correlations between relevant variables.

3.1.3 Population, interventions, comparisons, outcomes, and study designs (PICOS)

The PICOS model [96] has been utilized to methodically identify the fundamental elements of the studies that are part of the meta-analysis:

- **Population (P)**: Defining the intended study population in accordance with inclusion criteria, which may include characteristics related to health, demography, or other significant variables related to the research topic.
- **Interventions (I)**: Naming the particular treatments, interventions, or exposures that were examined in detail in all of the included research.

- **Comparisons (C)**: Comparing various intervention modalities or active treatment with placebo, for example, or comparing different study groups.

- **Outcomes (O)**: Highlighting the results or endpoints that are measured in the studies that are included; these may include clinical improvements, risk reductions, or other relevant metrics that are determined by the goals of the research.

- **Study Designs (S)**: Describing the various study designs that were used in the included articles and how they affected the strength and relevance of the combined evidence.

  This logical and planned technique guarantees openness in the selection of studies, based on carefully specified criteria, which in turn makes it easier to gather relevant evidence to fully address the research question that the meta-analysis poses.

3.2 Search Strategy

An essential part of every meta-analysis is the search strategy, which outlines the procedure for finding, locating, and choosing relevant studies to include. An effective search approach reduces selection bias, guarantees the meta-analysis's comprehensiveness and reproducibility, and strengthens the validity of the findings reached. The foundation of the search strategy is the development of a clear and targeted research question. By using the PICO (Population, Intervention, Comparator, Outcome) framework, the main elements of the study topic can be efficiently defined.

3.2.1 Databases to be searched

A complete collection of pertinent studies must be captured, which requires careful database selection. The main databases for scientific and

medical literature include Scopus, Web of Science, Embase, PubMed/MEDLINE, and the Cochrane Library. Different databases have different indexing terms and coverage, therefore search strategies must be customized for each database.

3.2.2 Search terms and keywords

In order to guarantee full coverage, a comprehensive list of search terms and keywords, including synonyms and variations, is required. In addition to restricted vocabulary phrases like Medical Subject Headings (MeSH) for PubMed, the search terms should include both free-text keywords and these terms. To efficiently combine search phrases and refine the results, use the Boolean operators AND, OR, and NOT.

3.3 Data Extraction

In a systematic review, data extraction is the procedure that takes place before to data analysis and following the identification of pertinent research. Getting the information needed to spot trends in the different studies that make up the review is the aim of data extraction in a meta-analysis [97].

3.3.1 Process for extracting data from included studies

In order to guarantee that reliable and pertinent data are collected from the included studies, the data extraction process in a meta-analysis is an essential part. This data serves as the basis for further data synthesis and analysis.

3.3.2 Variables to be extracted

The process of extracting data begins with the creation of a standardized data extraction form. The following sections of the form, which are not exclusive to them, must be included in order to fully personalize it and collect all pertinent data needed for the meta-analysis:

Study Identification (Title, authors, publication year, journal), Study Design(Type of study (e.g., randomized controlled trial, cohort study), setting, sample size), Population Characteristics(Demographics (age, sex, ethnicity), inclusion and exclusion criteria.), Interventions(Details of the treatments or interventions administered, including dosage and duration.) and Results(Numerical data for outcomes, measures of effect (e.g., risk ratios, odds ratios), and statistical significance.)

3.4 Statistical Analysis

Literature Review

A literature review is a summary of the body of published research on a subject. Its main academic function is to demonstrate comprehension and critical analysis of a certain subject. This aids in placing the current study into the framework of pertinent literature, improving readers' comprehension of the subject.

Many techniques, including argumentative, integrative, historical, methodological, systematic, or theoretical approaches, can be used to perform literature reviews. Clinical research is using more and more of these kinds of studies, particularly in epidemiology. One reason for their growing use is the necessity to evaluate small hazards that could have a big impact on public health or interest. They also make it possible to evaluate risk variables that are common in the general population in-depth. These approaches provide more precise effect estimates, boost statistical power, and enable the generalization of findings from individual research by determining if a factor is a risk both qualitatively and quantitatively.

Narrative reviews, meta-analyses of published data, pooled analyses, and prospectively planned pooled analyses are a few techniques for assembling evidence.

Prospero

A registry devoted exclusively to systematic reviews is called Prospero. It enables researchers to record the procedures of their systematic reviews and is run by the University of York. By registering a systematic review with Prospero, researchers can collaborate more easily, lower the possibility of duplicate research, and improve the transparency and caliber of systematic reviews. Comprehensive details on review goals, inclusion and exclusion standards, search tactics, and analytic techniques are included in the protocols filed in Prospero [98].

Open Science Framework

Not limited to systematic reviews, the Open Science Framework (OSF) is a platform that provides a more versatile framework for coordinating research projects across multiple disciplines. OSF provides tools to store, exchange and work together on protocols, data and other research resources. In addition to a plethora of other project types, it can be used to document research processes, including those for systematic reviews. Because of OSF's flexibility, researchers can more easily manage their projects and publish different phases of their work at different times [99]. Right in this platform, the abstract of the article on which this thesis is based is already available (DOI:10.17605/OSF.IO/3T6FK)

Cochrane Database of Systematic Reviews

Cochrane Database of Systematic Reviews (CDSR) is a premier resource for publishing high-caliber systematic reviews and is a component of the Cochrane Library. Cochrane, an international network of researchers, medical professionals, patients, and anyone with an interest in health

research, is responsible for its upkeep. Published in CDSR, systematic reviews undergo a rigorous peer review process and adhere to very precise Cochrane guidelines [100]. These guidelines were used in the writing of this thesis.

3.4.1 Meta-Analysis

Carefully considering whether to combine the numerical data of all or some research is an essential part of a systematic review. Meta-analysis produces an overall statistic (along with a corresponding confidence interval) from which the information needed to assess possible differences can be extrapolated. Among the possible advantages of meta-analyses are:

- **Improving Accuracy**: A large number of single studies are too tiny to provide strong proof of the impact of interventions. When an estimate is based on additional data, it is typically more accurate.
- **addressing issues that are not covered by a single study**: Primary research frequently include defined interventions and particular participants. It is feasible to test for consistency of effect over a larger range of groups and interventions and to look into the reasons behind variations in outcomes by analyzing trials with varied features.
- **Resolving disputes and generating new hypotheses**: Statistical synthesis of data makes it possible to evaluate the level of disagreement between studies and investigate the causes of different results.

Basic ideas form the foundation of common meta-analysis techniques: Meta-analysis is often carried out in two phases. Initially, for every trial, a summary statistic that consistently characterizes the intervention's impact is computed. For dichotomous data, one may use a hazard ratio,

and for continuous data, a difference between averages. The weighted average of the effects estimated in each individual study is used to create an overall estimate of the intervention effect in the second step. A weighted average is defined as:

$$\frac{\sum_{i=1}^{n} Y_i W_i}{\sum_{i=1}^{n} W_i}$$

W_i is the weight assigned to the i^{th} study, Y_i is the estimated intervention effect from the i^{th} study, and the summation is based on all of the studies. The weighted average and the average effect of the intervention coincide if all the weights are the same. A study's contribution to the overall average will rise with a larger weight applied to it.

The assumption that studies do not all estimate the same effect, but rather effects that follow a distribution, might be included when combining intervention effect estimates from several research. This forms the foundation of a meta-analysis with random effects. Alternatively, a fixed-effect meta-analysis is employed, provided that it is assumed that all studies estimate the same quantity.

The total effect standard error can be used to compute a confidence interval that demonstrates the accuracy of the estimate and to derive a P-value that illustrates the strength of the evidence against the null hypothesis that there is no effect.

All meta-analysis techniques can include an evaluation to ascertain if the variance in study results is random variation or whether it is significant enough to suggest inconsistent observed effects, in addition to providing a summary of the effect.

One of the many practical factors to be assessed in a meta-analysis is the issue of missing data. Authors should pay close attention to how missing data, such as those resulting from follow-up losses or analysis exclusions, affects the findings.

Graphs called Forest Plot are frequently used to illustrate meta-analyses. Effect estimates and confidence ranges for the individual stud-

ies and the meta-analysis are shown in the forest plot. A rectangle with a horizontal line extending to its sides that displays the point estimate of the intervention impact is used to symbolize each research. The meta-analysis's weight for the study is shown by the area of the rectangle, and the horizontal line shows the confidence interval, which is typically 95%. The confidence interval and the rectangle's area both offer significant information and contribute to our knowledge of the graph in distinct ways. The range of intervention effects that are consistent with the study's findings is shown by the confidence interval. The more weighted studies (often those with narrower confidence intervals) that have the most impact on the summary result, which is typically shown as a rhombus farther down the graph, are highlighted by the size of the diamond [101].

3.4.2 Measures of effect

A statistical technique for comparing the results of two intervention groups is an effect measure. These consist of mean differences, which compare mean values, and odds ratios, which measure the probability of an event. These measurements are separated into ratio measures, which are also referred to as absolute measures, and difference measures. One example of a ratio metric is the odds ratio, whereas a difference measure is the mean differences. Effect estimates use the variation in outcomes between groups to quantify the intervention's influence. A value of 1 for ratio measurements denotes no difference, and a value of 0 for difference measures denotes no change. Depending on the outcome's nature and the order of the interventions in the comparison, values above or below the "null" values may indicate a benefit or damage of the intervention. The precise effects of interventions are never known and can only be inferred from the research that are now accessible. An indication of uncertainty, such as a confidence interval or standard error (SE), should be included with any estimate [102].

Odds Ratio

A statistical tool for calculating the relationship between exposure to a risk factor and the desired outcome is the odds ratio (OR). Comparing the probability of an event between two groups is a common usage for it [102].

Odds is a measure that represents the ratio of the probability that an event will occur to the probability that it will not occur. Mathematically, for an event with probability p the odds is defined as:

$$Odds = \frac{p}{1-p}$$

Comparing the probability of an event between two groups is what the odds ratio (OR) does.

Consider a contingency table 2x2:

	Event (E+)	Non-Event (E-)
Group 1	a	b
Group 2	c	d

The odds for each group are calculated as follows:

- **Gruppo 1**: $\text{Odds}_1 = \frac{a}{b}$
- **Gruppo 2**: $\text{Odds}_2 = \frac{c}{d}$

The odds ratio is then defined as:

$$\text{OR} = \frac{\text{Odds}_1}{\text{Odds}_2} = \frac{a \cdot d}{b \cdot c}$$

Indicating that there is no difference between the groups is an odds ratio (OR) of 1. An increase in the probability of the occurrence in group 1 is indicated by an OR larger than 1, whereas a decrease in the probability of the event in group 1 is indicated by an OR less than 1.

For a number of reasons, the odds ratio is the favored metric in meta-analyses. It is resilient compared to other metrics since it is less impacted by inter-study variability. It is also adaptable and appropriate for data

from randomized clinical trials as well as case-control studies. Lastly, it makes it possible to aggregate the findings of several studies to derive an estimate of the overall effect.

Per l'OR, l'intervallo di confidenza (IC) al 95% è calcolato con la formula:

$$\text{IC} = \exp\left(ln(\text{OR}) \pm z \cdot \text{SE}\right)$$

Where:

For statistical symmetry and normalcy in the OR distribution, we employ the logarithm. z is equal to 1.96, which corresponds to a 95% confidence level, is obtained from the standard normal distribution. It means that, in actuality, we anticipate that, 95% of the time, the true value of the OR will fall within this range. SE is the standard error of the logarithm of the OR, and is equal to:

$$\text{SE} = \sqrt{\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}}$$

The IC gives a range of values that correspond to the genuine odds ratio. The correlation is statistically significant if the confidence interval (CI) excludes 1. In OR estimate, a smaller range denotes higher precision and a larger range, higher uncertainty. By giving a clear view of the OR estimate's variability and dependability, the CI aids in understanding how robust the estimate is.

Effects Models

Studies are weighted in meta-analyses based on how accurate their estimations are, usually in an inverse relation to variation. Statistical models with fixed effects are used to assume a common effect across research, while models with random effects allow for variation across studies by assuming different effects to be estimated in each investigation [103].

- **Fixed Effects Model** Each estimate y_i obtained from the i-th study is assumed by the fixed-effects model to reflect a realization from a single normal population with mean θ. We base our model

on the assumption that there is only one true parameter in reality, which is determined by estimating it from every study. As a result, it is assumed that all research come from a single, homogeneous population with no variation. But each estimate has an inherent variability, represented by s_i^2.

$$y_i \sim \mathcal{N}(\theta, s_i^2), \text{ for } i = 1, \ldots, k$$

where k is the number of studies.

The average of the estimates derived from the various research, weighted by the inverse of their variance, is the meta-analytic estimate. Assume k studies exist. If the measure is OR the effect measure is represented as y_i on a logarithmic scale.

$$\hat{\theta} = \frac{\sum_{i=1}^{k} w_i y_i}{\sum_{i=1}^{k} w_i}$$

where $w_i = \frac{1}{s_i^2}$ is the weight associated with the study i, that is, the reciprocal of the variance of the study estimate, k is the number of studies included in the meta-analysis, and y_i represents the point estimate.

- **Random Effects Model** We presume that the estimates from the k studies may have come from two or more distinct populations when heterogeneity arises. Getting an overall estimate that takes into account and integrates this heterogeneity across populations is the aim. Each unique effect estimate, y_i, has a distribution derived from a normal population with variance $\tau^2 + s_i^2$ and mean μ:

$$y_i \approx \mathcal{N}(\mu, \tau^2 + s_i^2)$$

It is feasible to get an overall estimate that takes this variability into account when there is heterogeneity. It makes intuitive sense to use a function of Q to estimate the variance τ^2 given that Q is a

measure of heterogeneity among k studies (DerSimonian & Laird).

$$\tau^2 = \max\left(0, \frac{Q-(k-1)}{\sum_{i=1}^{k} w_i - \frac{\left(\sum_{i=1}^{k} w_i^2\right)}{\sum_{i=1}^{k} w_i}}\right)$$

where:

$$w_i^* = \frac{1}{s_i^2 + \tau^2}$$

Summary odds ratio

The Odds Ratios (OR) of multiple studies that are part of a meta-analysis are combined to get the pooled estimate (sOR). This measure takes data from several studies into account to produce a general assessment of the relationship between an exposure and an outcome.

Using the 2x2 contingency table for each study, the odds ratios for all the studies that are part of the meta-analysis are first calculated in order to get the summary odds ratio. Next, for each research, the standard error (SE) of the logarithm of the OR is determined.

The precision of the studies is then used to determine their weight. In the final estimate, studies with a lower standard error—which denotes better precision—are weighted higher. Either a fixed-effects model or a random-effects model can be used to combine the ORs. While the random-effects model takes into account the likelihood of fluctuation in the effect between research, the fixed-effects model assumes that all studies are measuring the same genuine effect.

The pooled estimate (sOR) for the random-effects model can be computed using the exponential of the weighted average of the logarithms of the individual study odds ratios. The equation is:

$$\text{sOR} = \exp\left(\frac{\sum_i w_i \ln(\text{OR}_i)}{\sum_i w_i}\right)$$

where w_i represents the weight of the study i and OR_i is the odds ratio of the study i.

Lastly, the combined estimate of the logarithm of the OR and its aggregate standard error are used to produce the confidence interval for

the summary odds ratio using the following formula:

$$\mathrm{IC} = \exp\left(\ln(\mathrm{sOR}) \pm z \cdot \mathrm{sSE}\right)$$

where z is the crucial value for the intended confidence interval, and sSE is the standard error of the logarithm of the combined OR. Compared to ORs from individual research, the summary odds ratio offers a more comprehensive and robust measure of connection. It also aids in evaluating how consistently effects are observed in different research and identifying any noteworthy variability.

3.4.3 Assessment of Heterogeneity

It is inevitable that included research in a systematic review will differ from one another. Heterogeneity is the term used to describe variation among research in a systematic review. Making the distinction between various forms of heterogeneity is helpful. Clinical diversity, also referred to as clinical heterogeneity, is the variation in the subjects, treatments, and results of studies. Conversely, methodological diversity (also known as methodological heterogeneity) refers to variations in study design, outcome measuring instruments, and bias risk. The variety in apparent intervention effects across trials, known as statistical heterogeneity, can be attributed to either clinical or methodological variation, or to both. When the intervention's observed effects vary more than what would be predicted by random error alone, statistical heterogeneity takes place [101].

Evaluating the level of coherence between the included research' findings is crucial. In general, statistical heterogeneity is evident if there is little overlap in the confidence ranges for the various study results, which are typically graphically depicted with horizontal lines. More technically, heterogeneity can be found using a particular statistical test, the χ^2-test [101].

Certain academics contend that statistical heterogeneity is unavoid-

able in meta-analyses due to the constant presence of clinical and methodological variety. Because heterogeneity will always exist whether or not it can be discovered by a statistical test, the test for heterogeneity may not be the deciding factor in the analysis that is chosen. In order to overcome this, techniques that quantify study inconsistency have been developed, changing the emphasis from merely looking for heterogeneity to evaluating how it affects the meta-analysis [101]. One helpful statistic to measure this kind of inconsistency is:

$$I^2 = \left(\frac{Q - (k-1)}{Q} \right) \times 100$$

where:

$$Q = \sum_{i=1}^{k} w_i (y_i - \hat{\theta})^2$$

It follows an approximate χ^2_{k-1} distribution. If the p-value is less than 0.10, the homogeneity null hypothesis is rejected due to the underpowered nature of this test.

Limits for evaluating I^2 may be deceptive, since the importance of discrepancy relies on multiple variables. The following serves as a broad framework for interpretation when it comes to meta-analyses of randomized trials:

- From 0% to 40%: inconsistency may not be significant.
- From 30% to 60%: may indicate moderate heterogeneity.
- From 50% to 90%: may indicate substantial heterogeneity.
- From 75% to 100%: represents substantial heterogeneity.

The magnitude and direction of the effects as well as the degree of the evidence of heterogeneity both affect the relevance of the observed value of I^2. For instance, the confidence interval for I^2 or the p-value from the chi-square test can be used to determine significance. A low number of included research increases the uncertainty in the value of I^2 [101].

Methods for dealing with Heterogeneity

Review authors should take statistical heterogeneity into account when interpreting data, particularly if the effect's direction varies. There are various methods available if heterogeneity is discovered among a set of papers that would otherwise be appropriate for a meta-analysis [101].

- **Re-check that the data are correct**. Significant heterogeneity may be a sign of mistakes made during data entry or extraction in the meta-analysis program. Incorrectly entering standard errors as standard deviations in continuous data, for instance, can result in excessively narrow confidence intervals and inadequate overlap, which can significantly increase variability. Heterogeneity can also be caused by errors pertaining to the units of analysis.

- **Don't do a meta-analysis**. Meta-analytical content is not required in a systematic review. Citing an average number for the intervention's effect could be misleading if the findings show significant variation, especially if the effect's direction is inconsistent.

- **Explore heterogeneity**. The reasons for the variation in study results are obviously of interest. This procedure is difficult since there are frequently a lot of differentiable features to pick from across studies. Using meta-regression or subgroup analysis, heterogeneity can be investigated. Only studies that are fully pre-specified prior to reviewing study results can yield reliable conclusions, and even these conclusions need to be evaluated cautiously. At best, investigations of heterogeneity that are developed after heterogeneity has been discovered can result in the formulation of theories. They should normally not be included in a review's conclusions and should be interpreted much more cautiously. Furthermore, it is dubious to conduct heterogeneity research in situations when there are few studies.

- **Ignore heterogeneity**. Meta-analyses using fixed effects disregard heterogeneity. Generally speaking, the best estimate of the intervention impact is the summary effect estimate from a fixed-effect meta-analysis. But the presence of heterogeneity raises the possibility that there are multiple intervention effects rather than just one. As a result, the fixed effect summary estimate can represent an intervention effect that isn't really present in any population, which would result in an excessively narrow and nonsensical confidence interval.

- **A Random effects meta-analysis should be conducted**. Heterogeneity among studies can be incorporated using a random-effects meta-analysis. This does not replace a careful examination of heterogeneity. It is mostly meant for inexplicable heterogeneity.

- **Examine the effect size again**. Heterogeneity could be a deliberate result of selecting the wrong effect measure. For instance, extreme heterogeneity may be visible when the mean difference is utilized, but not when the most appropriate standardized mean difference is used, when studies gather continuous outcome data using several scales or units. Furthermore, the degree of variability among results for dichotomous outcomes may depend on the effect measure selected. Specifically, homogeneous odds ratios or risk ratios will inevitably result in heterogeneous risk differences and vice versa when the risks of the reference group differ. It is unclear, nevertheless, if selecting these indicators should be based on the homogeneity of the intervention effect in a given meta-analysis.

- **Exclude studies**. One or two peripheral studies with results that differ from the rest of the research could be the cause of heterogeneity. Excluding studies from a meta-analysis on the basis of their findings is generally a bad idea because it can induce bias. However, the study might be dismissed with more confidence if a

clear explanation for the peripheral finding is found. This criterion is inaccurate because it is too easy to meet, as each meta-analysis for each study often has at least one attribute that sets it apart from the others. It is advisable to conduct analyses as part of a sensitivity analysis with and without peripheral research. Whenever feasible, the protocol need to include information on likely sources of clinical variability that might result in these kinds of circumstances.

3.4.4 Publication Bias

Publication bias arises from the observation that studies reporting statistically significant are published more frequently than studies that report no association between exposure and the event of interest [103, 104]. The likelihood of a study being published is often related to the statistical significance of the results, with the most significant results being more likely to be published. This leads to publication bias in meta-analyses of published studies. Detecting such bias is critical, as it can lead to erroneous conclusions in systematic reviews [105, 106].

Selection models are the basis of one class of techniques for detecting publication bias. These techniques often construct estimating approaches that take into account the publication process and express it using weighted distribution theory [107]. Unfortunately, the complexity of selection models frequently restricts their usefulness. Furthermore, these models incorporate weight functions in an attempt to account for publication bias; nevertheless, they typically rely on strong assumptions that are challenging to validate. Because of this, the validity of corrected results may be questioned; hence, these methods are typically employed in sensitivity assessments [105, 106].

The usage of the funnel plot is the foundation of another class of techniques for identifying publication bias. Typically, this graph displays effect sizes in relation to their accuracies, or the inverse of standard er-

rors. The funnel plot typically exhibits distortion when publication bias is present. Examining the asymmetry of the funnel plot can help one intuitively determine publication bias, albeit this kind of visual evaluation is frequently arbitrary. Numerous statistical methods, including Egger's regression test and its expansions and Begg's rank test, have been proposed to address this problem [108, 109]. A substantial association indicates the existence of publication bias. The rank test examines the relationship between effect sizes and the matching sample variances. Regression of standardized effect sizes versus accuracy is done by Egger's test, where the regression intercept should be zero if publication bias is not present. A weighted regression of the effect sizes against their standard errors, weighted by the inverse of their variances, is equal to this regression, it should be emphasized; in the absence of publication bias, the slope of the weighted regression should be zero instead of the intercept [105, 110].

Chapter 4

Results

This section will present the results of the research carried out by exploiting the methodological knowledge mentioned in the previous chapters. In particular, all data processing steps that were necessary for the completion of statistical analysis will be described, thus reporting their interpretation.

4.1 Study Selection

To ensure that the papers included were only the most relevant, a strict and methodical process was used to select the studies. There were multiple steps in the process: The selection of studies was first made by establishing the inclusion and exclusion criteria necessary for the purpose of our investigation. In particular:

Inclusion Criteria

The literature search had to be carried out keeping in mind that the articles to be viewed had to relate to:

- The therapies of patients with advanced melanoma.
- The Highlighting of the data obtained concerning the sex of patients admitted.

- The Patients toxicities encountered during the treatment.

- In addition, it was decided not to consider time constraints on the publication of the studies to be included.

Exclusion Criteria

Instead, exclusion criteria were evaluated during and at the end of the article screening process:

- "Case Report" studies were excluded. These studies in fact reported too low a number of patients to make a comparison between the sexes of patients.

- Studies with lack of stratification of data related to patients' sex or related to their adverse events. Indeed, such lack of stratification does not allow measurement of the association of patients' sex with treatment-related toxicities.

Sources

The second step was to conduct the literature search. Two databases were tapped for this search, from which a combination of the studies found was made so that as many articles as possible could be viewed, and then included in order to have a sufficiently large amount of data to statistical analyses. The Databases in question are PubMed and Embase, which were consulted by means of two search strings [1], which differed in syntax, obviously using the same keywords. The syntax is found to be different between the two strings because each database requires a different query language. As for the keywords, it is possible to say that they were carefully chosen according to the inclusion criteria.

[1] The strings used for the search can be found in Chapter : Supplementary Material

Screening Process

Once we have made a collection of the studies found thanks to the properly created research strings, a Screening phase of each article was needed. That moment was extremely important for the purpose of our investigation, as the data needed was extracted. This procedure was carried out under the supervision of several collaborators to avoid involving random errors in statistical analysis. Studies had to provide sufficient information to estimate the odds ratio and confidence intervals at 95% (i.e. they had to publish odds ratios (OR) or raw data) for the number of patients experiencing both adverse events and non-adverse events. In addition, the studies had to be independent in order to avoid giving double weight to some studies. In particular, in order to implement data extraction as effectively as possible, we removed duplicates and revised titles and abstracts for a first study filter. Furthermore, the entire text had to be analyzed to verify its relevance to the criteria established, thus excluding studies that did not comply with them.

The primary objective of the search was to extrapolate generic toxicities (irAEs) from the various articles. During the screening process, however, it was possible to realize that many studies reported only specific toxicities such as dermatological, thyroid, regarding the pituitary gland, ocular, regarding the liver, gastrointestinal, grade III-IV, and regarding the kidneys. Therefore we decided to continue to develop the research by also studying the toxicities listed above .

4.1.1 Flow Chart of study selection process

PRISMA 2020 Flow Chart [111] for new systematic reviews which included searches of databases and registers only [4.1]:

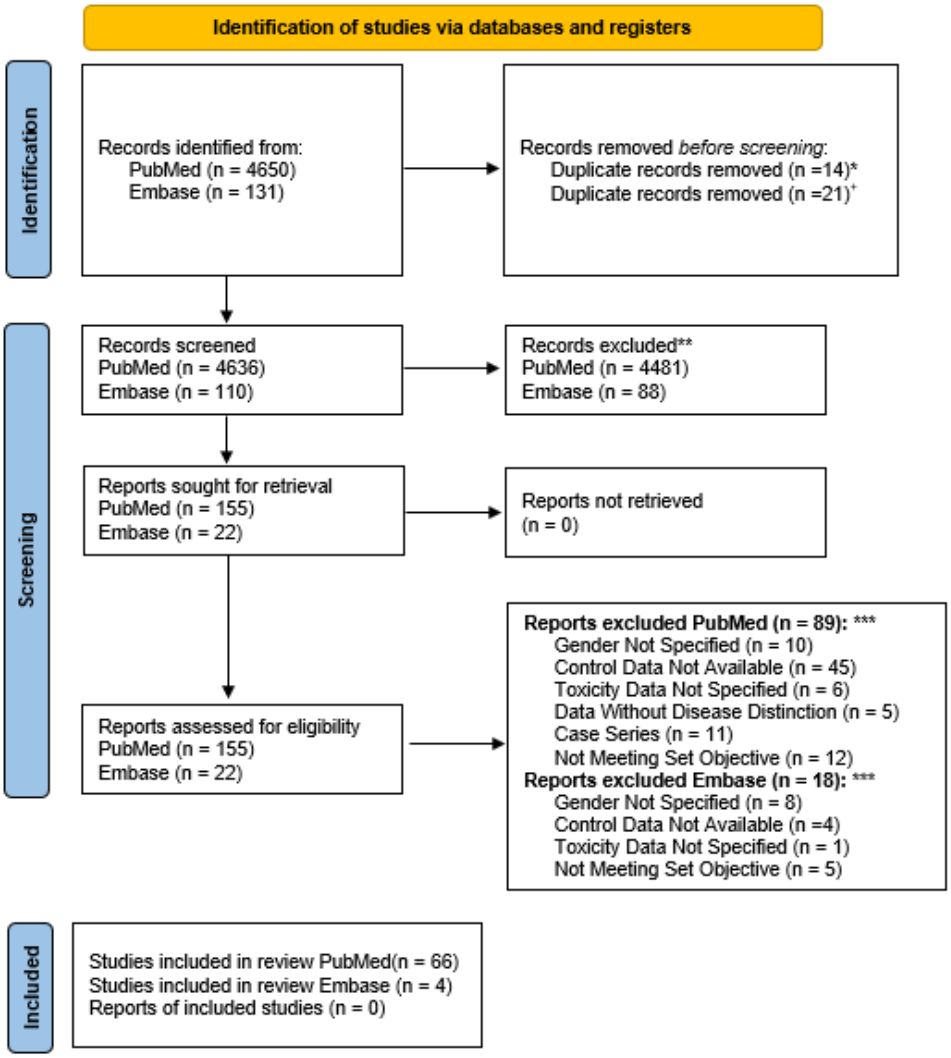

Figure 4.1

Flow Chart Description

** STRING 1 + STRING 2;*

+ In common with the research on PubMed;

*** Sum of articles excluded for various reasons (No stratification by sex , review, meta-analysis...);*

**** Sex Not Specified:There is no sex reference in patients with or without AEs;*

Control Data Not Available: there is a sex stratification, only for patients with AEs, whereas there is no stratification for non-AEs;

Toxicity Data Not Specified: there is no reference to sex-elated to the

various toxicities (AEs);

Data Without Disease Distinction: The patients present may present other than Melanoma, and the data does not report it when the sex is indicated;

Case Series: Case reports or series of few patients;

Not Meeting Set Objective: It does not relate to the objective set.

In total, 70 studies were included in the meta-analysis. The selected studies cover a wide range of articles concerning the response to oncological treatments of patients with advanced melanoma relative to the sex of the patient, allowing an in-depth analysis of the variables under consideration.

4.2 Study Characteristics

To collect information from each study, a questionnaire was created with the purpose of developing a table useful for statistical analysis. The questionnaire included, for each article: the type of adverse event in patients, the author, the year of publication, the country, the study design, the type of study, the type of therapy used, the number of total patients, the number of patients having adverse events distinctly by sex, and finally the number of patients not having adverse events distinctly by sex.

This showed that the studies considered came from virtually all parts of the world: Europe, USA, Canada, Japan and Australia. We find, however, a prevalence of studies from Europe and the USA. It is also possible to note that there is a prevalence of retrospective cohort studies. Only 9 trials and 5 prospective cohort studies. The time frame of the studies considered also is defined from 2015 to 2024.

Better specifics, on each study ([112–188]), are given in Table 1 [4.1]. These tables present the data, for each study, extracted during the last "screening" phase. Specifically, the type of therapy utilized in the treatment, year of publication, first author of the study, country of origin,

study design, type of study, total number of patients, number of male and female patients with the corresponding AE, and finally the number of male and female patients without the corresponding AE are reported for each study, according to the reference AEs.

AEs	THERAPY TYPE	YEAR OF PUBLICATION	FIRST AUTHOR	COUNTRY	STUDY DESIGN	DETAILS	N° OF PATIENTS	N° AEs (M/F)	N° No AEs (M/F)
Dermatological AEs	IMMUNO	2017	Nakamura et al.	Japan	Observational Study	Retrospective - Cohort Study	35	4/5	14/12
	IMMUNO	2019	Quach et al.	USA	Observational Study	Retrospective - Cohort Study	318	74/46	128/70
	IMMUNO	2020	Bottlaender et al.	France	Observational Study	Retrospective - Cohort Study	189	22/17	87/63
	IMMUNO	2020	Nakano et al.	Japan	Observational Study	Retrospective - Cohort Study	128	34/27	38/31
	IMMUNO	2021	Dousset et al.	France	Observational Study	Ambispective - Cohort Study	457	57/28	205/167
	IMMUNO	2021	Park et al.	Korea	Observational Study	Retrospective - Cohort Study	1711	30/17	1131/533
	IMMUNO	2021	Salinas et al.	France	Observational Study	Retrospective – Cohort Study	181	12/13	80/76
	IMMUNO	2021	Van der Kooij et al.	Netherlands	Observational Study	Retrospective - Cohort Study	1454	22/12	884/536
	IMMUNO	2022	Ai-Tram et al.	USA	Observational Study	Retrospective - Cohort Study	235	69/58	73/35
	IMMUNO	2022	Gullo et al.	Italy	Observational Study	Retrospective - Cohort Study	146	38/27	48/35
	IMMUNO	2022	Patel et al.	USA	Observational Study	Retrospective - Cohort Study	155	58/25	44/30
	TT	2013	Degen et al.	Germany	Clinical Trial	Phase II - NCT00823402	55	18/23	11/3
	TT	2015	Sinha et al.	UK	Observational Study	Retrospective - Cohort Study	107	3/9	56/39
	TT	2021	Kähler et al.	Germany	Observational Study	Retrospective - Cohort Study	422	53/61	180/128
	TT	2022	Gullo et al.	Italy	Observational Study	Retrospective - Cohort Study	140	17/16	67/40
	TT+IMMUNO	2022	Shreberk-Hassidim et al.	Israel	Observational Study	Retrospective - Cohort Study	95	24/10	39/22
Hypophysitis	IMMUNO	2014	Alexander et al.	USA	Observational Study	Retrospective - Cohort Study	154	15/2	84/53
	IMMUNO	2015	Min et al.	USA	Observational Study	Retrospective - Cohort Study	187	16/6	102/63
	IMMUNO	2017	Brilli et al.	Italy	Observational Study	Prospective - Cohort Study	273	4/5	103/161
	IMMUNO	2018	De Sousa et al.	Australia	Observational Study	Retrospective - Cohort Study	46	8/3	26/11
	IMMUNO	2019	Snyders et al.	USA	Observational Study	Retrospective - Cohort Study	117	10/5	64/38
	IMMUNO	2019	Wei et al.	Scotland	Observational Study	Retrospective - Cohort Study	51	4/2	34/11

Table 4.1: *Extracted data for each study grouped by type of AEs*

(continued on next page)

AEs	THERAPY TYPE	YEAR OF PUBLICATION	FIRST AUTHOR	COUNTRY	STUDY DESIGN	DETAILS	N° OF PATIENTS	N° AEs (M/F)	N° No AEs (M/F)
Gastrointestinal AEs	IMMUNO	2016	O'Connor et al.	UK	Observational Study	Retrospective - Cohort Study	83	10/7	40/26
	IMMUNO	2019	Abu-Sbeih et al.	USA	Observational Study	Retrospective - Cohort Study	346	117/66	105/68
	IMMUNO	2020	Nahar et al.	Australia	Observational Study	Retrospective - Cohort Study	1507	80/41	960/501
Kidney AEs	IMMUNO	2021	Stein et al.	France	Observational Study	Retrospective - Cohort Study	239	18/23	109/89
	TT	2016	Teuma et al.	France	Observational Study	Retrospective - Cohort Study	74	33/11	12/18
	TT	2017	Teuma et al.	France	Observational Study	Retrospective - Cohort Study	38	5/4	15/14
	TT	2021	Abdelrahim et al.	USA	Observational Study	Retrospective - Cohort Study	1864	47/25	1051/541
	TT	2022	Seethapathy et al.	USA	Observational Study	Retrospective - Cohort Study	199	21/21	90/67
Liver AEs	IMMUNO	2018	Brandon et al.	USA	Observational Study	Retrospective - Cohort Study	218	12/5	122/79
	IMMUNO	2021	Smith et al.	Canada	Observational Study	Retrospective - Cohort Study	63	22/10	19/12
	IMMUNO	2021	Van der Kooij et al.	Netherlands	Observational Study	Retrospective - Cohort Study	1454	124/73	782/475
	TT	2021	Biewenga et al.	Netherlands	Observational Study	Retrospective - Cohort Study	3111	87/52	1749/1223
	TT	2021	Van der Kooij et al.	Netherlands	Observational Study	Retrospective - Cohort Study	1077	41/38	573/425
Ocular AEs	IMMUNO	2021	Dimitriou et al.	Switzerland	Observational Study	Retrospective - Cohort Study	304	3/1	185/115
	TT	2017	De la Cruz-Merino et al.	USA	Clinical Trial	Phase III - coBRIM NCT01689519	495	39/31	247/178
	TT	2021	Dimitriou et al.	Switzerland	Observational Study	Retrospective - Cohort Study	186	4/3	109/70
	TT+IMMUNO	2021	Eikenberry et al.	USA	Observational Study	Retrospective – Cohort study	34	10/4	11/9
Thyroid irAEs	IMMUNO	2018	De Filette et al.	Belgium	Observational Study	Prospective - Cohort Study	99	5/12	31/51
	IMMUNO	2018	Guaraldi et al.	Italy	Observational Study	Prospective - Cohort Study	52	16/6	14/16
	IMMUNO	2018	Yano et al.	Japan	Observational Study	Retrospective - Cohort Study	24	2/5	9/8
	IMMUNO	2020	Olsson-Brown et al.	UK	Observational Study	Retrospective - Cohort Study	103	5/11	52/35
	IMMUNO	2021	Muir et al.	Australia	Observational Study	Retrospective - Cohort Study	1246	308/212	518/210

Table 4.1: *Extracted data for each study grouped by type of AEs*

(continued on next page)

AEs	THERAPY TYPE	YEAR OF PUBLICATION	FIRST AUTHOR	COUNTRY	STUDY DESIGN	DETAILS	N° OF PATIENTS	N° AEs (M/F)	N° No AEs (M/F)
Other AEs	IMM + TT	2021	Samlowski et al.	USA	Observational Study	Retrospective - Cohort Study	23	13/10	4/1
	IMMUNO	2015	Tirumani et al.	USA	Observational Study	Retrospective - Cohort Study	147	25/21	63/38
	IMMUNO	2017	Fujimura et al.	Japan	Clinical Trial	Phase I - UMIN000020222	9	2/1	4/2
	IMMUNO	2018	Fujimura et al.	Japan	Observational Study	Prospective - Cohort Study	46	9/13	15/9
	IMMUNO	2018	Uhara et al.	Japan	Clinical Trial	Phase IV - EPPV study	95	9/4	43/39
	IMMUNO	2019	Bisschop et al.	Netherlands	Observational Study	Retrospective - Cohort Study	147	11/15	68/55
	IMMUNO	2019	Bisschop et al.	Netherlands	Observational Study	Retrospective - Cohort Study	147	40/40	37/30
	IMMUNO	2019	Duma et al.	USA	Observational Study	Retrospective - Cohort Study	245	68/80	80/37
	IMMUNO	2019	Okada et al.	Japan	Observational Study	Retrospective - Cohort Study	15	3/5	1/6
	IMMUNO	2019	Sachpekidis et al.	Germany	Observational Study	Prospective - Cohort Study	16	5/5	4/2
	IMMUNO	2019	Schadendorf et al.	Austria	Clinical Trial	phase II - CheckMate 172 - NCT02156804	1008	53/31	504/420
	IMMUNO	2019	Wei et al.	Scotland	Observational Study	Retrospective - Cohort Study	51	11/4	27/9
	IMMUNO	2020	Eggermont et al.	Multicenter European	Clinical Trial	Phase III - EORTC 1325/KEYNOTE-054 - NCT02362594	1011	117/73	505/316
	IMMUNO	2020	Helgadottir et al.	Sweden	Observational Study	Retrospective - Cohort Study	19	3/2	7/7
	IMMUNO	2020	Hopkins et al.	Different Countries	Pooled Analysis	BRIM-2, BRIM-3 and coBRIM clinical trials	962	64/86	501/311
	IMMUNO	2020	Nardin et al.	France	Observational Study	Retrospective - Cohort Study	111	33/28	25/25
	IMMUNO	2020	Suo et al.	Canada	Observational Study	Retrospective - Cohort Study	186	47/41	62/36
	IMMUNO	2020	Watson et al.	Canada	Observational Study	Retrospective - Cohort Study	492	137/61	183/111
	IMMUNO	2020	Zhao et al.	China	Observational Study	Retrospective - Cohort Study	93	32/22	22/17

Table 4.1: *Extracted data for each study grouped by type of AEs*

(continued on next page)

AEs	THERAPY TYPE	YEAR OF PUBLICATION	FIRST AUTHOR	COUNTRY	STUDY DESIGN	DETAILS	N° OF PATIENTS	N° AEs (M/F)	N° No AEs (M/F)
Other AEs	IMMUNO	2021	Klee et al.	Germany	Observational Study	Retrospective - Cohort Study	9	3/2	3/1
	IMMUNO	2021	Mesti et al.	Slovenia	Observational Study	Retrospective - Cohort Study	99	18/20	37/24
	IMMUNO	2021	Reschke et al.	Germany	Observational Study	Prospective - Cohort Study	17	5/3	4/5
	IMMUNO	2021	Sema-Higuita et al.	Germany	Observational Study	Retrospective - Cohort Study	319	70/41	122/86
	IMMUNO	2021	Sema-Higuita et al.	Germany	Observational Study	Retrospective - Cohort Study	319	107/62	85/65
	IMMUNO	2021	van der Kooij et al.	Netherlands	Observational Study	Retrospective - Cohort Study	1454	230/131	676/417
	IMMUNO	2022	Cybulska-Stopa et al.	Poland	Observational Study	Retrospective - Cohort Study	585	79/71	263/172
	IMMUNO	2022	Kudura et al.	Switzerland	Observational Study	Retrospective - Cohort Study	103	24/13	50/16
	IMMUNO	2022	Uhara et al.	Japan	Clinical Trial	Phase IV – Post -Marketing Study	2008	645/602	385/376
	IMMUNO	2023	Müller et al.	Switzerland	Observational Study	Retrospective - Cohort Study	20	14/5	1/0
	IMMUNO	2023	Truong et al.	Canada	Observational Study	Retrospective - Cohort Study	71	16/6	24/25
	IMMUNO	2024	Christensen et al.	Denmark	Observational Study	Retrospective - Cohort Study	454	38/63	221/134
	TT	2016	Bjoern et al.	Denmark	Clinical Trial	Phase I	10	3/0	5/2
	TT	2018	Ben-Betzalel et al.	Israel	Observational Study	Retrospective - Cohort Study	78	5/5	41/27
	TT	2020	Nakamura et al.	Japan	Observational Study	Retrospective - Cohort Study	10	2/3	2/3
	TT	2021	van der Kooij et al.	Netherlands	Observational Study	Retrospective - Cohort Study	1077	56/55	558/408
	TT	2021	van der Kooij et al.	Netherlands	Observational Study	Retrospective - Cohort Study	1077	124/115	490/348

Table 4.1: *Extracted data for each study grouped by type of AEs*

4.3 Analysis results

From the variables in the above table, created by means of a special questionnaire, it was possible to create additional datasets, one per type of adverse event. These sets are characterized on the rows by the study under consideration, and on the columns by the name of the author of the study, the OR, the Upper 95% Confidence Interval, the Lower 95% Confidence Interval, and the number of patients. It is important to note that in this case the OR calculation was performed, not to measure the efficacy or effect of a given treatment, but to assess the association of patients' sex with toxicities due to the treatments undergone. Specifically, if the OR determined is greater than 1 and statistically significant then a positive association can be inferred for female patients. This association implies a higher probability of occurrence of adverse events in women than in men.

All statistical computations were performed using R (version 4.3.0). Once the data set was set up, characterized by the data extracted from the various studies, through appropriate statistical techniques the following results could be deduced:

4.3.1 Overall result

In most cases, the difference between males and females regarding toxicity of oncology treatments for advanced melanoma is not significant. The table [4.2] shows, for each type of AE, the summary OR estimates with its confidence intervals and the value of heterogeneity. From this table is possible to see that only for adverse events concerning the thyroid is possible to see a relevant difference between male and females (sOR= 2.00, 95%CI [1.41-2.85]). From this initial analysis, we infer that a difference between males and females is present, to the detriment of females, regarding the toxicity of cancer treatments for advanced melanoma. This means that a female patient treated for advanced melanoma is almost

two times more likely to experience an adverse thyroid event than a male patient.

AEs type	sOR	IC	I^2
irAE	1.05	0.88-1.24	25.65
Grade III-IV	1.14	0.85-1.53	69.01
Dermatological	1.22	0.97-1.53	48.18
Gastrointestinal	0.87	0.66-1.16	0
Hypophysitis	0.7	0.41-1.19	0
Kidney	0.9	0.47-1.72	70.91
Liver	0.95	0.78-1.16	0
Ocular	1.18	0.89-1.59	0
Thyroid	2	1.41-2.85	29.39

Table 4.2: *Summary results applied to each AEs group, with raw ORs, 95% Interval Confidance and heterogeneity*

For a more in-depth analysis let's look carefully at the value, for each group, the heterogeneity (I^2). Given the values 48%, regarding the "Dermatological AEs" group, 69%, of the "Grade III-IV" group, and finally 70%, regarding the "Kidney AEs" group, it is possible to deduce the need to conduct an in-depth heterogeneity analysis. Using the "Leave-one-out" method, we obtain the results shown by tebella [4.3]. It is possible to see that the estimates became statistically significant for dermatologic adverse events (sOR= 1.31, 95%CI[1.06-1.61]), again to the disadvantage of women compared to men as previously in the case of thyroid adverse events.

Regarding the publication bias given the values shown in the table, it

AEs type	sOR	IC	I^2
irAE	1.05	0.88-1.24	25.65
Grade III-IV	1.01	0.82-1.25	30.28
Dermatological	1.31	1.06-1.61	34.83
Gastrointestinal	0.87	0.66-1.16	0
Hypophysitis	0.7	0.41-1.19	0
Kidney	1.2	0.86-1.69	0
Liver	0.95	0.78-1.16	0
Ocular	1.18	0.89-1.59	0
Thyroid	2	1.41-2.85	29.39

Table 4.3: *Summary results applied to each AEs group, with raw ORs, 95% Interval Confidance and heterogeneity already assessed*

can be inferred that, distinctly for each group characterized by the side effect, there is an evidance of absence of publication bias because the $p - values$, given from Egger's test and Begg's test, are non-significant values, as shown in table [4.4].

AEs type	Egger's test (p-value)	Begg's test (p-value)
irAE	0.90	0.64
Grade III-IV	0.94	0.60
Dermatological	0.76	1
Gastrointestinal	0.81	1
Hypophysitis	0.71	0.27
Kidney	0.36	0.48
Liver	0.55	0.81
Ocular	0.22	0.48
Thyroid	0.68	1

Table 4.4: *Publication Bias for each AEs group*

For all the remaining groups, distinguished by type of adverse event, it can be concluded that the sex of the patient, treated for advanced melanoma with oncologic therapies such as immuno and target therapy, is not significant in triggering toxicities.

4.3.2 Thyroid AEs

Patients, distinctly by sex, treated with oncology therapies such as target therapy and immunotherapy have significant differences in the occurrence of thyroid adverse events (sOR=2.00, 95%CI [1.41-2.85], I^2=29.39%) to the disadvantage of females.

A better comprehension about the single studies the table [4.5] is reported. This table shows, for each study on thyroid AEs, the ORs with the corresponding values of the extremes of the confidence intervals.

Also a better comprehension about the overall outcome the forest plot [4.2] is reported [2].

[2] "Obs" stands for Observational; "RCT" stands for Randomized Clinical Trials.

Author (Year)	OR	Lower 95% CI	Upper 95% CI
De Filette (2016)	1.45	0.46	4.53
Yano (2018)	2.81	0.42	18.73
Olsson-Brown (2020)	3.26	1.04	10.22
Muir (2021)	1.70	1.34	2.16
Christensen (2024)	2.88	1.81	4.58
Guaraldi (first line) (2018)	2.66	0.46	15.25
Guaraldi (second line) (2018)	0.35	0.06	2.01

Table 4.5: *Summary results applied to each study for thyroid AEs analysis, with raw ORs, upper and lower 95% Interval Confidance*

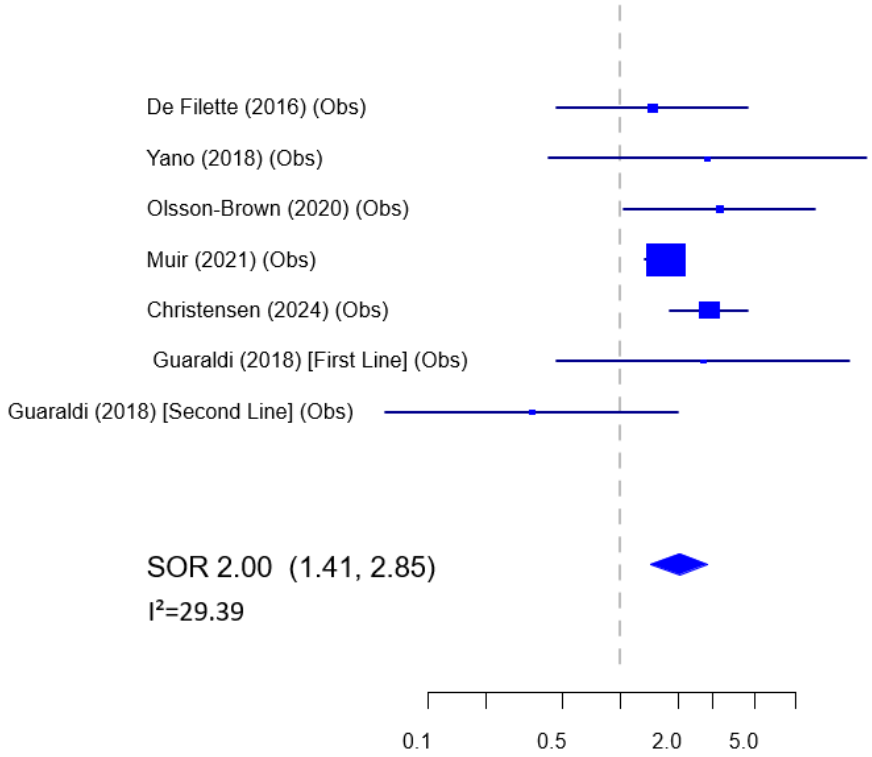

Figure 4.2: *Forest Plot of ORs for studies about Thyroid adverse events*

From this forest plot, it can be seen that sex difference affects the occurrence of thyroid adverse events due to advanced melanoma treatments to the disadvantage of females.

Heterogeneity

Given the I^2= 29.39% value, it is possible to infer that inconsistency may not be significant. From this result, it would be irrelevant to further investigate the heterogeneity of the studies.

Publication Bias

Regarding "Publication Bias" in this context, the result obtained is not statistically significant (Egger's test= 0.68, Begg's test= 1). From which an evidance of absence of Publication Bias can be inferred as demonstrated by the funnel plot [4.3].

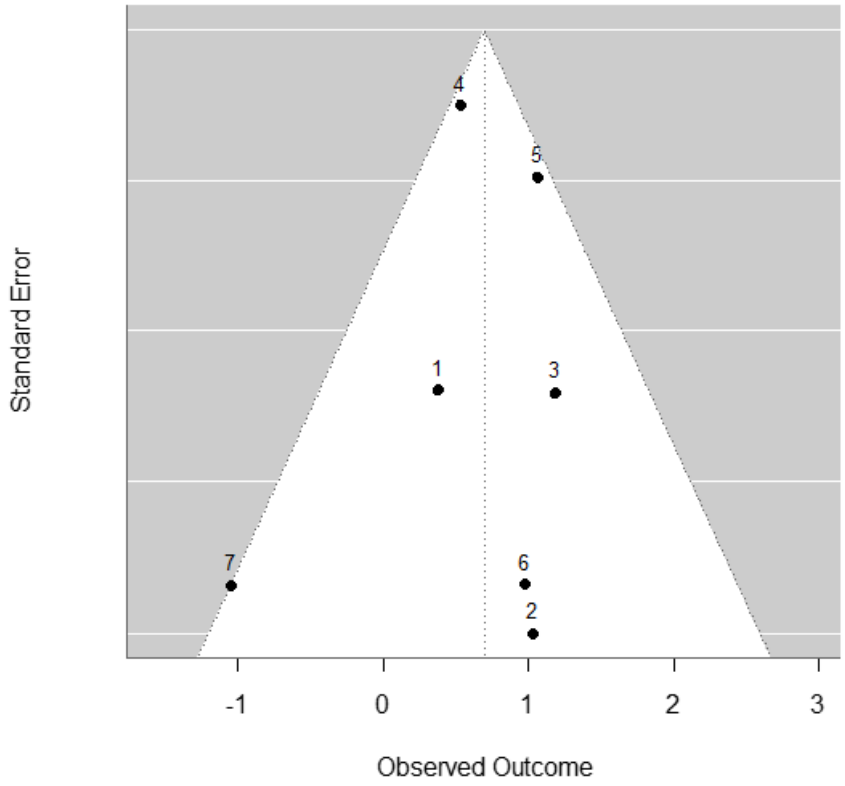

Figure 4.3: *Funnel Plot of studies about thyroid adverse events*

4.3.3 Dermatological AEs

Patients, distinctly by sex, treated with oncology therapies such as target Therapy and immunotherapy have no significant differences in the occurrence of dermatological adverse events (sOR=1.22, 95%CI [0.97-1.53], I^2=48.18%).

A better comprehension about the single studies the table [4.6] is reported. This table shows, for each study on dermatological AEs, the ORs with the corresponding values of the extremes of the confidence intervals.

Also a better comprehension about the overall outcome the forest plot [4.4] is reported.

Author (Year)	OR	Lower 95% CI	Upper 95% CI
Degen (2013)	4.68	1.13	19.34
Sinha (2015)	4.30	1.09	16.93
Bjoern (2016)	0.41	0.01	12.65
Nakamura (2017)	1.45	0.31	6.69
Quach (2019)	1.13	0.71	1.81
Hopkins (2020)	2.16	1.52	3.08
Nakano (2020)	0.92	0.45	1.85
Bottlaender (2020)	1.06	0.52	2.17
Park (2021)	1.20	0.65	2.19
Salinas (2021)	1.14	0.48	2.65
Dousset (2021)	0.60	0.36	0.99
Kähler (2021)	1.61	1.05	2.49
Van der Kooij(2021)	0.89	0.44	1.83
Shreberk-Hassidim (2022)	0.73	0.29	1.82
Patel (2022)	0.65	0.33	1.26
Ai-Tram (2022)	1.75	1.02	2.98
Gullo (Arm IMMUNO) (2022)	1.02	0.53	1.99
Gullo (Arm TT) (2022)	1.57	0.71	3.46

Table 4.6: *Summary results applied to each study for dermatological AEs analysis, with raw ORs, upper and lower 95% Interval Confidence*

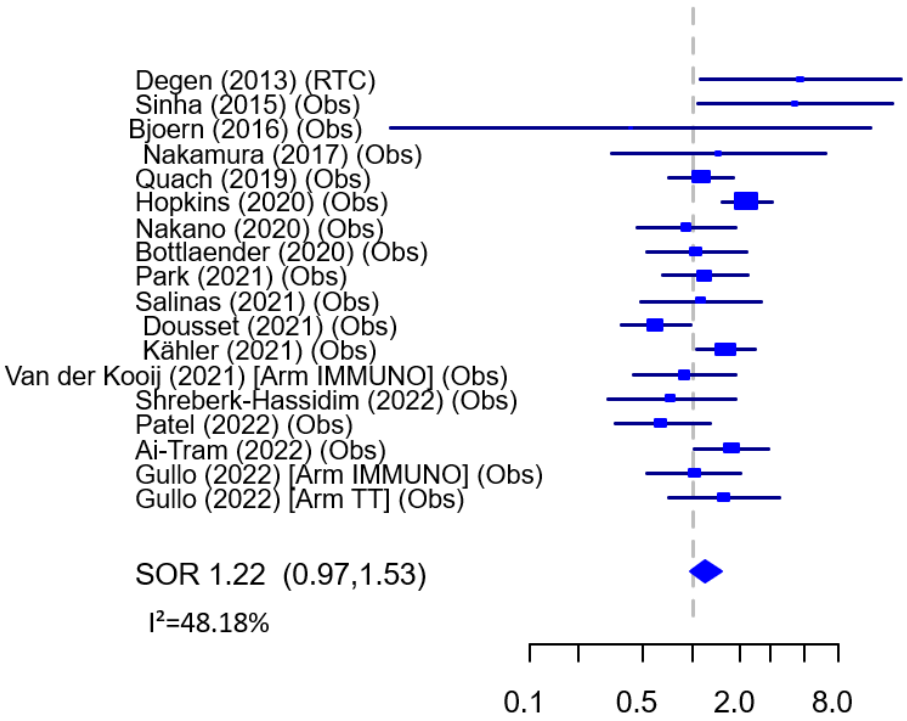

Figure 4.4: *Forest Plot of ORs for studies about dermatological adverse events*

From this forest plot, it can be seen that sex difference does not affect the occurrence of dermatological adverse events due to advanced melanoma treatments.

Heterogeneity

Given the I^2= 48.18% value, it is possible to infer that may indicate moderate heterogeneity. From this result, it would be relevant to further investigate the heterogeneity of the studies. To do this was conducted to opt for the "Leave-one-out" method. Without Dousset's study, heterogeneity drops to 34.83%. We also see that the value of the estimates increases making them statistically significant. This approach shows a significant difference between males and females to the disadvantage of females regarding the occurrence of dermatological adverse events due to treatment of advanced melanoma (sOR= 1.31, 95%CI[1.06-1.61]).

Publication Bias

Regarding "Publication Bias" in this context, the result obtained is not statistically significant (Egger's test= 0.76, Begg's test= 1). From which an evidance of absence of Publication Bias can be inferred as demonstrated by the funnel plot [4.5].

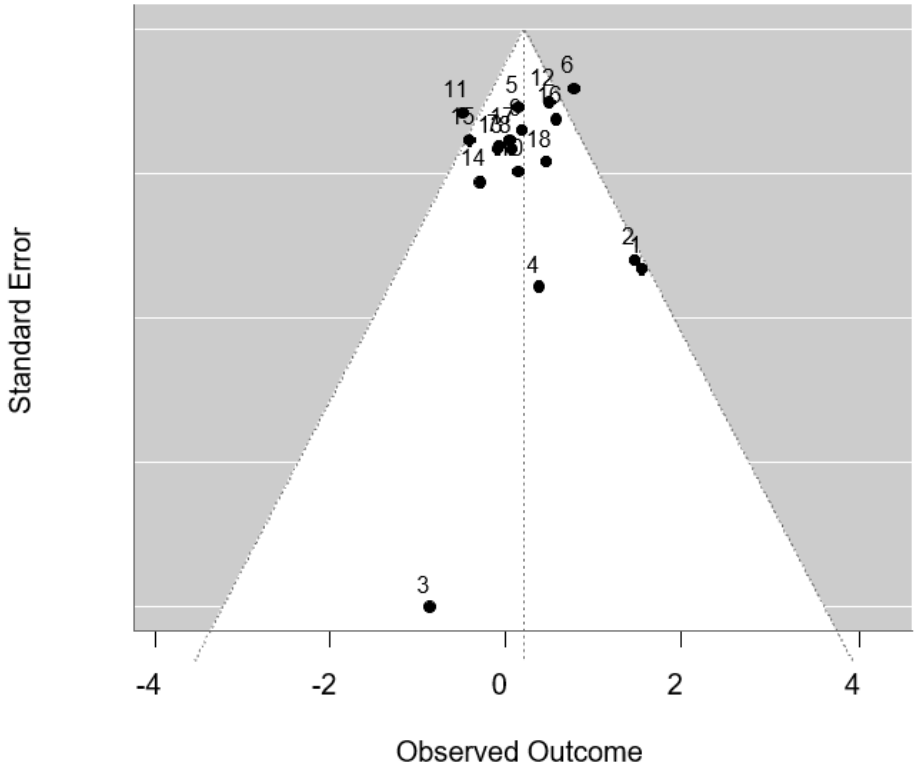

Figure 4.5: *Funnel Plot of studies about dermatological adverse events*

4.4 Subgroup analysis results

Subgroup analyses could provide important information on variables that may influence treatment toxicity. Such an analysis is a technique used in meta-analyses to explore differences in effects between distinct groups within included studies. This approach allows investigation of whether the overall treatment effect varies according to specific characteristics. In this research, in particular, we wanted to conduct a more in-depth analysis regarding the line of treatment (first and second line), the type

of therapy (Target therapy and Immunotherapy or Mono and Combo) and finally the type of the study considered.

4.4.1 Line of Treatment

First-line treatment in this context refers to the initial course of action decided upon following the diagnosis. When a tumor recurs or does not respond to first-line therapy, it is referred to as second-line therapy. It covers complementary or supplementary therapies to first-line treatment.

Thyroid AEs

Subgroup analysis showed that there is not a difference in toxicity between the sexes depending on the line of treatment:

A consonant and in-depth analysis does not show a difference, in toxicity to treatment type, between the two sexes. In fact, from a meta-regression it is possible to say that there is no statistically significant difference between the sexes with respect to treatment type ($p - value = 0.44$). It might be useful to investigate other variables that might influence the results, such as study quality, effect variability, and other possible modifiers of impact. Indeed, in this case it can be seen that there are only 3 second-line studies with high heterogeneity.

4.4.2 Mono vs. Combo

Mono therapy, referred to as "Mono", is the use of only one medication or treatment. Combining medications or treatments to improve therapeutic efficacy is referred to as "Combo".

Grade III-IV AEs

Subgroup analysis showed that there is not a difference in toxicity between the sexes depending on the type of treatment (mono vs combo):

A consonant and in-depth analysis does not show a difference, in toxicity to treatment type, between the two sexes. In fact, from a meta-regression it is possible to say that there is no statistically significant difference between the sexes with respect to treatment type ($p - value = 0.97$). It might be useful to investigate other variables that might influence the results, such as study quality, effect variability, and other possible modifiers of impact.

Thyroid AEs

Subgroup analysis showed that there is not a difference in toxicity between the sexes depending on the type of treatment (mono vs combo):

A consonant and in-depth analysis does not show a difference, in toxicity to treatment type, between the two sexes. In fact, from a meta-regression it is possible to say that there is no statistically significant difference between the sexes with respect to treatment type ($p - value = 0.08$). It can be seen, however, that this statistic is not significant at 5% but is significant at 10%. By this it is possible to say that there may be little difference between the two subgroups. It might be useful to investigate other variables that might influence the results, such as study quality, effect variability, and other possible modifiers of impact.

As mentioned earlier, regarding adverse events concerning the thyroid, female patients are significantly more likely than men to experience side effects during treatment regardless of treatment type.

Dermatological AEs

Subgroup analysis showed that there is not a difference in toxicity between the sexes depending on the type of treatment (mono vs combo):

A consonant and in-depth analysis does not show a difference, in toxicity to treatment type, between the two sexes. In fact, from a meta-regression it is possible to say that there is no statistically significant difference between the sexes with respect to treatment type ($p - value =$

0.22). Investigating other variables that could affect the outcomes, such as study quality, effect variability, and other possible impact modifiers, could be helpful.

4.4.3 Immuno vs. Target therapy

This subsection will show the significant results obtained from the analysis of subgroups characterized by terget-therapeutic vs. immuno-therapeutic treatment.

Grade III-IV AEs

Treatment-related subgroup analysis showed that there is a difference in toxicity between the sexes depending on the type of treatment (immunotherapy vs target therapy):

A consonant and in-depth analysis shows a difference, in toxicity to treatment type, between the two sexes. In fact, from a meta-regression it is possible to say that there is statistically significant difference between the sexes with respect to treatment type ($p-value$= 0.003). From these results, a divergence between female and male patients can be identified when the therapy exploited for the treatment of advanced melanoma is target therapy. Thus, with the latter type of treatment, women are more likely to experience a severe adverse event than men.

Dermatological AEs

Treatment-related subgroup analysis showed that there is a difference in toxicity between the sexes depending on the type of treatment (immunotherapy vs target therapy):

A consonant and in-depth analysis shows a difference, in toxicity to treatment type, between the two sexes. In fact, from a meta-regression it is possible to say that there is statistically significant difference between the sexes with respect to treatment type ($p-value$= 0.001). From these results, a divergence between female and male patients can be identified

when the therapy exploited for the treatment of advanced melanoma is target therapy. Thus, with the latter type of treatment, women are more likely to experience a dermatological adverse event than men.

4.4.4 Study type

This subsection presents the results obtained from the subgroup analysis related to the type of study considered for the analyses (Observational Retrospective vs. Observational Prospective vs. Trials). This type of analysis could only be done for the included studies dealing with general adverse events. This is because all the remaining studies, included in the meta-analysis, are retrospective observational studies and therefore it was not possible to do such a subgroup analysis for the remaining studies dealing with the other types of adverse events.

Generic AEs

Study type-related subgroup analysis showed that there is not a difference in toxicity between the sexes depending on the type of the studies (Observational Retrospective vs. Observational Prospective vs. Trials):

A consonant and in-depth analysis does not show a difference, in toxicity to study type, between the two sexes. In fact, from a meta-regression it is possible to say that there is not statistically significant difference between the sexes with respect to study type ($p-value= 0.52$). It might be useful to investigate other variables that might influence the results, such as study quality, effect variability, and other possible modifiers of impact. Indeed, in this case it can be seen that there are only 3 Trials and 3 Observational Prospective studies over the 24 total studies included.

Chapter 5

Discussion

Many patients' prognosis have improved as a result of the substantial changes made to the treatment of melanoma by immunotherapy and targeted medicines. Adverse events (AEs), or side effects, are a common consequence of these therapies and can make it difficult for patients to continue receiving them. Studies have indicated that women may be more susceptible to side effects from cancer treatments, especially cytotoxic ones. The purpose of this study is to investigate how sex may affect the likelihood of these unfavorable events by conducting a meta-analysis. Data on sex and toxicity was gathered from numerous publications. With a summary odds ratio of 2.00 (95%CI[1.41–2.85]) the data showed that women are double more likely to experience thyroid-related adverse events (AEs) than men, with no between-study heterogeneity (I^2=29%). This indicates that the likelihood of them experiencing these side effects is double that of males. Also is possible to say that there was little variability (I^2=29%). Given the non-statistically significant estimates, the effects of severe toxicities (grade III–IV) or those involving the skin, gastrointestinal tract, pituitary, kidney, liver, or eyes were not significantly impacted by sex. Women receiving targeted therapy (TT) had a greater incidence of dermatologic adverse events ($p-value$= 0.001) and severe AEs ($p-value$= 0.003) compared to men. It is possible to say that adverse events (AEs) connected to the thyroid are more

common in women. They also exhibit a higher risk of serious adverse events and dermatological problems mainly during targeted treatments. From these results obtained it is possible to conclude that, during oncological treatments for advanced melanoma, it is necessary to pay more attention to female patients as they are more likely to develop side effects during therapy. In particular, it is important to monitor this type of patient by assessing symptoms related to dermatological, thyroid and severe side effects. This meta-analysis highlights a topic that has not received much attention in the literature: the possible differences in adverse events caused by anticancer therapy for patients with advanced melanoma between males and females. It is among the first to examine this possibility. The findings point to a new line of investigation for future study: being female may have an impact on cancer treatment by favoring the occurrence of its adverse effects in comparison to being male. Research on advanced melanoma treatments indicates that there is a sex difference in the toxicity of cancer treatments, which is harmful to women, despite the dearth of direct investigations in this field. Our findings are indirectly supported by these similarities. The accuracy of our results is guaranteed by the strict technique we used, which included a big sample and accurate measurements. This work creates fresh opportunities, urging additional investigation to validate and develop these preliminary results. Not just in terms of advanced melanoma treatment, but also in reference to cancer treatments generally. The debate of how sex affect the emergence of treatment-related toxicities is enriched and broadened by this thesis. It emphasizes how crucial it is to take these things into account when evaluating how well patients are responding to treatment. This investigation could lead to more precise and personalized medicine, but more importantly, more risk-aware therapies by determining the best treatment for each sex. This analysis provides valuable information that health care providers can use to tailor treatment plans and improve outcomes for patients of both sexes.

5.1 Strengths and Limitations

Choosing a meta-analysis is one of the best options for an appropriately precise study. These analyses summarize the overall assessments by highlighting the key findings of a particular topic on which multiple investigations have been carried out. This statistical analysis has advantages and disadvantages like any other. One strength of the research is statistical power, which comes from combining multiple studies, no matter how tiny, to provide an analysis that is more accurate and persuasive. Such studies also contribute to the resolution of conflicting topics in the scientific literature by offering a more thorough synthesis of the existing data. The investigation of variability among the many research is another strength. This allowed for the identification of feature subgroups that moderate treatment toxicity across studies. In this type of research setting, limiting variables might have included potential measurement errors, such as misallocation during data extraction, or random errors that could have been created and compromised the clinical studies' internal validity. Furthermore, it's probable that the authors of the studies that made up the articles focused mostly on reporting the result that was the most positive and suppressed other results that were not statistically significant. Finally, the main limitations of this study could be caused during the extraction of data from the studies to form a data set on which to perform the various statistical analyses. One is that our research follows a retrospective approach. The second is that from most of the articles it was not possible to extrapolate all data of interest, i.e., generic adverse events and generic non-adverse events by sex, because these articles reported only one specific adverse event that occurred in the study patient cohort. For example, out of a total of 200 male patients and 150 female patients, 10 males and 15 females had a dermatological adverse event. With this, it was not possible for us to tell whether the remaining patients, differentiated by sex, developed other adverse events. However, this type of issue guided us toward the analysis of subgroups

differentially by type of adverse event. In this way we were able to turn this limitation into a very useful and meaningful insight.

In conclusion, it is possible to say that given the strengths and despite the limitations, this meta-analysis is definitely a solid foundation and a solid starting point on which to continue more and more in-depth and interesting research in the field of advanced melanoma treatment.

Chapter 6

Conclusions

This thesis contributes to further debate on how the sex of patients with advanced melanoma influences the development of toxicities related to cancer treatments. The analysis conducted shows that women have a higher risk of developing thyroid-related adverse events. In addition, women show a higher likelihood of experiencing dermatological and grade III-IV adverse events when receiving targeted therapies (TT) than when receiving immunotherapy. These findings could lead to a deeper understanding of the risks of cancer therapies in terms of the adverse events they can cause. That said, it is of paramount importance to carefully monitor female patients in order to ensure adequate adherence to cancer treatment so that they can intervene as effectively as possible by detecting early signs of any thyroid, dermatological, and severe side effects.

Given the importance that the research topic and its results focus on, it would be very interesting to learn more about the main causes that differentiate the probability of occurrence of adverse events, due to treatments for advanced melanoma, between the two sexes. In fact, the purpose of such research could be extended not only in for this context but for additional oncological and other contexts.

Chapter 7

Supplementary Material

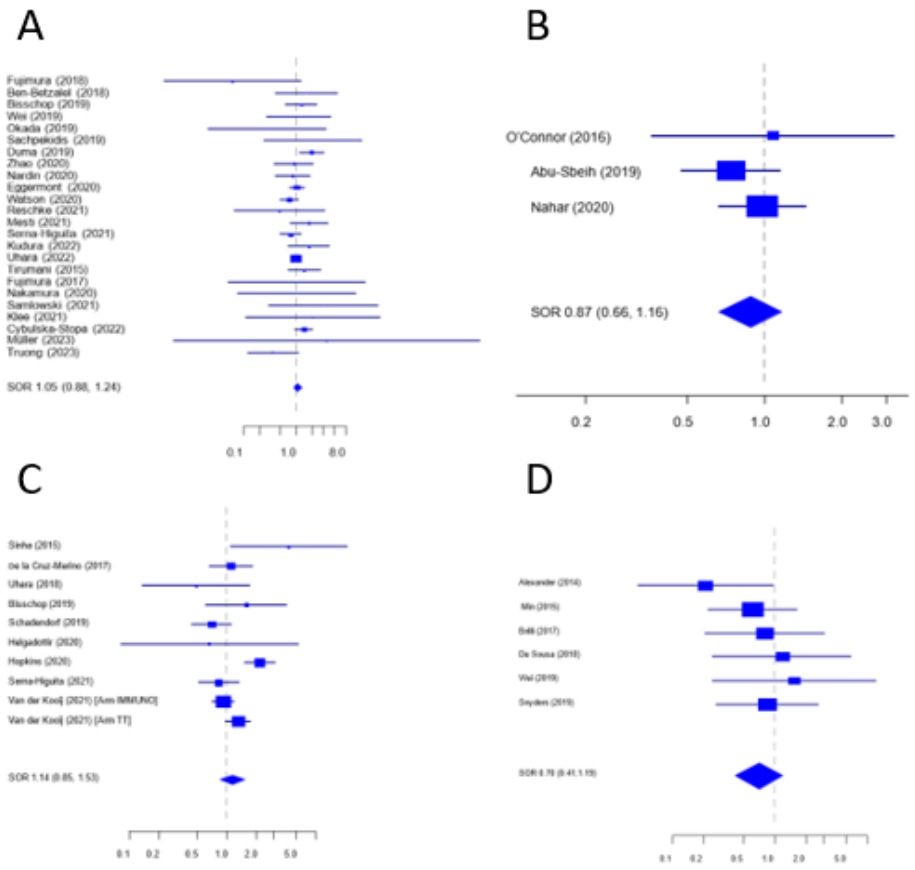

Figure 7.1: *A: Forest Plot for generic AEs; B: Forest Plot for Gastrointestinal AEs; C: Forest Plot for Severe AEs; D: Forest Plot for Hypophysitis AEs*

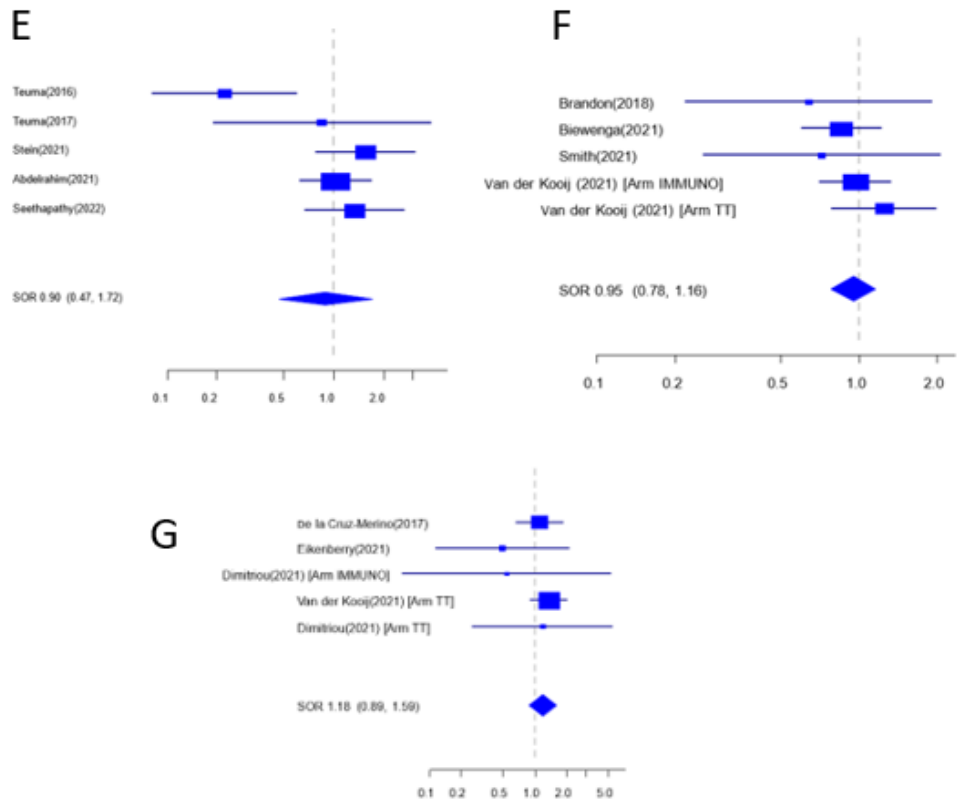

Figure 7.2: *E: Forest Plot for Kidney AEs; F: Forest Plot for Liver AEs; G: Forest Plot for Ocular AEs*

Strings

As mentioned earlier, the literature search, to find the articles selected for analysis, was conducted on both PubMed and Embase. This survey was supported by experienced librarians. With this assistance, the following two strings were generated:

- **PubMed**: *((((Immunotherapy OR "immuno-therap*" OR immunotherapeutic* OR "immune therapy" OR "immune therapies" OR "Molecular Targeted Therapy"[Mesh] OR "targeted therap*" OR "targetted therap*" OR "targeted treatment*" OR "targetted treatment*" OR target*[TI] OR ("Immune Checkpoint Inhibitors"[Mesh]) OR "Immune Checkpoint Inhibitors" [Pharmacological Action] OR "im-*

mune checkpoint inhibit" OR "Chemotherapy, Adjuvant"[Mesh] OR Adjuvant OR "neoadjuvant therapy"[MeSH] OR Neoadjuvant OR "CTLA-4 Antigen/antagonists and inhibitors"[Mesh] OR "anti-Cytotoxic T Lymphocyte Associated Antigen 4" OR Anti-CTLA4 OR "Anti-CTLA-4" OR "CTLA4 inhibitor*" OR "CTLA-4 inhibitor*" OR "CTLA4 antagonist*" OR "CTLA-4 antagonist*" OR "Programmed Cell Death 1 Receptor/antagonists and inhibitors"[Mesh] OR "anti pd1" OR "anti-pd1" OR "anti pdl1" OR "anti-pdl-1" OR "anti-programmed cell death-1" OR "anti-programmed cell death ligand-1" OR "PD1 inhibitor*" OR "PD-1 antagonist*" OR "PD-1 inhibitor*" OR "PDL1 inhibitor* OR "PDL-1 inhibitor* OR "PDL1 antagonist* OR "PDL-1 antagonist* OR "Proto-Oncogene Proteins B-raf/antagonists and inhibitors"[Mesh] OR "BRAF inhibitor*" OR "BRAF-MEK inhibitor*" OR "LAG-3 inhibitor*" OR "LAG-3 antagonist*" OR "LAG3 inhibitor*" OR "LAG3 antagonist*" OR "Lymphocyte Activation Gene 3 Protein"[Mesh] OR Nivolumab OR Pembrolizumab OR Ipilimumab OR Atezolizumab OR Relatlimab OR Avelumab OR Durvalumab OR Vemurafenib OR Dabrafenib OR Trametinib OR Cobimetinib OR Binimetinib OR Encorafenib) OR (("programmed cell death-1" OR "programmed cell death ligand-1") AND (antagonist OR inhibitor))) OR ("Cytotoxic T Lymphocyte Associated Antigen 4" AND (antagonist OR inhibitor))) AND ("adverse effects"[MeSH Subheading] OR poisoning [MeSH Subheading] OR toxicity [MeSH Subheading] OR "adverse effect" OR "adverse effects" OR "adverse event" OR "adverse events" OR "side effect" OR "side effects" OR trae OR Fatigue OR cough OR nausea OR appetite OR constipation OR Arthralgia OR arthralgic OR "joint pain" OR "joint pains" OR diarrhea OR diarrhoea OR (infusion* AND react*) OR "autoimmune reactions" OR autoimmunity OR "allergic reaction" OR hypersensitivity OR fever OR chills OR shivering OR flushing OR rash OR*

"exanthema"[MeSH Terms] OR "exanthema"[All Fields] OR itching OR dizzy OR dizziness OR vertigo OR wheezing OR Dyspnea OR dyspnoea OR breathless OR "breathing difficult*" OR "difficulty breathing" OR "short of breath" OR "shortness of breath" OR weakness OR frailty OR headache OR vomit OR vomiting OR nausea OR " low blood pressure" OR hypotension OR dermatitis OR vitiligo OR lichen* OR "Lichen Planus"[Mesh] OR thyroiditis OR colitis OR hepatitis)) AND (melanoma)*

- **Embase**: *('immunotherapy/exp' OR 'immunotherapy' OR 'immunotherap*' OR 'immunotherapeutic*' OR 'immune therapy' OR 'immune therapies' OR 'molecularly targeted therapy/exp/mj' OR 'targeted therap*' OR 'targetted therap*' OR 'targeted treatment*' OR 'targetted treatment*' OR 'immune checkpoint inhibitor/exp' OR 'immune checkpoint inhibitors' OR 'immune checkpoint inhibit*' OR 'adjuvant chemotherapy/exp' OR 'adjuvant' OR 'neoadjuvant chemoradiotherapy/exp' OR 'neoadjuvant' OR 'cytotoxic t lymphocyte antigen 4/exp' OR 'anti-cytotoxic t lymphocyte associated antigen 4' OR 'anti ctla4' OR 'anti-ctla-4' OR 'ctla4 inhibitor*' OR 'ctla-4 inhibitor*' OR 'ctla4 antagonist*' OR ('ctla-4 antagonist* programmed cell death 1 receptor/antagonists' AND 'inhibitors') OR 'anti pd1' OR 'anti-pd1' OR 'anti pdl1' OR 'anti-pdl-1' OR 'anti-programmed cell death-1' OR 'anti-programmed cell death ligand-1' OR 'pd1 inhibitor*' OR 'pd-1 antagonist*' OR 'pd-1 inhibitor*' OR 'pdl1 inhibitor*' OR 'pdl-1 inhibitor*' OR 'pdl1' OR 'pdl-1' OR (('b raf kinase/exp' OR 'b raf kinase') AND ('drug antagonism/exp' OR 'drug antagonism')) OR ('cytotoxic t lymphocyte associated antigen 4' AND ('antagonist' OR 'inhibitor'))) AND ('poisoning' OR 'toxicity' OR 'adverse effect' OR 'adverse effects' OR 'adverse event' OR 'adverse events' OR 'side effect' OR 'side effects' OR 'trae' OR 'fatigue' OR 'cough' OR 'appetite' OR 'constipation' OR 'arthralgia' OR 'arthralgic' OR 'joint pain' OR 'joint*

pains' OR 'diarrhea' OR 'diarrhoea' OR ('infusion' AND 'react*') OR 'autoimmune reactions' OR 'autoimmunity' OR 'allergic reaction' OR 'hypersensitivity' OR 'fever' OR 'chills' OR 'shivering' OR 'flushing' OR 'rash' OR 'rash'/exp OR 'exanthema' OR 'itching' OR 'dizzy' OR 'dizziness' OR 'vertigo' OR 'wheezing' OR 'dyspnea' OR 'dyspnoea' OR 'breathless*' OR 'breathing difficult*' OR 'difficulty breathing' OR 'short of breath' OR 'shortness of breath' OR 'weakness' OR 'frailty' OR 'headache' OR 'vomit' OR 'vomiting' OR 'nausea' OR 'low blood pressure' OR 'hypotension' OR 'dermatitis' OR 'vitiligo' OR 'lichen*' OR 'lichen planus'/exp OR 'thyroiditis' OR 'colitis' OR 'hepatitis') AND ('sexual characteristics'/exp OR 'sex characteristic*' OR 'sex difference*' OR 'sex':ti OR 'sexes':ti) AND ('melanoma')*

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