A Review on Combinatorial Therapies in Melanoma

By

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Declaration

It is hereby declared that

1. The project submitted is my own original work while completing degree at Brac University.
2. The project does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The project does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

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Ethics Statement

The study does not involve any kind of animal or human trial.

Abstract

Melanoma is the most deadly form of skin cancer due to its resistance to traditional cytotoxic treatment. However, innovative treatments have changed this disease's clinical trajectory. Understanding cancer microenvironment interaction and tumor oncogenesis led to these breakthroughs. Targeting the oncogenic mitogen-activated protein kinase (MAPK) pathway, notably BRAF, and MEK, improves overall and progression-free survival for BRAF-mutant melanoma. Furthermore, recent studies have shown long-lasting responses in a number of cancers after treatment with inhibitors of the immune suppressive programmed cell death 1 receptor (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) pathways to stimulate an anti-tumour immune response. Results from treating melanoma with immune-modulating and targeted therapies are promising. There may be further improvement potential when these drugs are combined. In this review, I will discuss current immunotherapies and targeted medications, as well as the results of combination studies and possible future therapeutic approaches.

Keywords: Melanoma, CLTA-4, Combination therapy, ipilimumab, nivolumab.

Dedication

I dedicate this work to everyone who inspired me in my work and especially to my supervisor.

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List of Acronyms

AE: Adverse events

APC: Antigen-presenting cells

BRAF: B-Raf protein

BRAFi: BRAF inhibitor

cM: Cutaneous Melanoma

CR: Complete response

CTLA-4: Cytotoxic T lymphocyte-associated antigen 4

ERK: Extracellular signal-regulated kinase

FDA: Food and Drug Administration

HR: Hazard ratio

ICI: immune Checkpoint Inhibitor

ICB: Immune checkpoint blocker

MITF: Microphthalmia transcription factor

MEDS: automated melanoma diagnosis system

MAPK: Mitogen-activated protein kinase

MHC: Membrane histocompatibility complex

mTOR: Mammalian target of rapamycin

NR: Not reported

ORR: Objective response rate

OS: Overall survival

PD-1: Programmed cell death 1

PD-L1: Programmed cell death 1 ligand 1

PFS: Progression-free survival

PI3K: Phosphoinositide 3-kinase

PR: Partial response

RT: Radiation therapy

SCC: Squamous cell carcinoma

TERT: Telomerase reverse transcriptase

TCR: T cell receptor

UV: Ultraviolet radiation

V600: Amino acid substitution at position 600 in BRAF from valine

V600E: Amino acid substitution at position 600 in BRAF from valine to glutamic acid

V600K: Amino acid substitution at position 600 in BRAF from valine to lysine.

Chapter 1

1.1 Introduction

Melanocytes, the cells responsible for the skin's pigmentation, are the origin of melanoma. It is the deadliest form of skin cancer because it can spread rapidly to other places of the body if detected and treated late (Melanoma Skin Cancer | Understanding Melanoma, n.d.). The American Cancer Society claims that melanomas arise when melanocytes' DNA is broken, causing uncontrolled cell growth and division. Which results from heredity, a compromised immune system, and UV radiation from the sun and tanning beds, among other things (Leonardi et al., 2018). Melanoma can develop anywhere on the skin, even in places like the palms of the hands, the soles of the feet, and under the fingernails that are not exposed to sunlight. Additionally, melanocyte-containing tissues like the eyes, mouth, and other areas of the body might develop it (Elder et al., 2020). Early identification and treatment of melanoma are essential for improving outcomes and raising survival rates (Sboner et al). Melanoma can be treated in a variety of ways, including surgery, radiation therapy, chemotherapy, immunotherapy, and targeted therapy, depending on the patient's preferences and the cancer's location and stage (Melanoma Treatment (PDQ®)–Patient Version - NCI, n.d.).

Combination therapy is frequently used to treat melanoma because it might enhance results and raise the possibility of a favorable response to therapy. Combination therapy has been demonstrated to increase overall survival, progression-free survival, and response rates in patients with advanced melanoma, according to a review paper in the Journal of Clinical Oncology (Hodi et al., 2016) . Combination therapy has the benefit of assisting in the reduction of drug resistance (Zhou & Johnson, 2018). Using numerous medications that target various pathways can make it more challenging for cancer cells to adapt and build resistance, as the same review article states, as opposed to how frequently cancer cells can develop resistance to

single-agent therapy (Hodi et al., 2016). Additionally, combining various medicines, including as chemotherapy or radiotherapy and immunotherapy, can enhance the efficacy of treatment. Combining chemotherapy with immunotherapy can help "overcome immune resistance, enhance the activity of immune effector cells, and increase the efficacy of both modalities (Krattinger et al., 2021).

Due to its potential to increase treatment effectiveness and prevent drug resistance, the drug combination is used to treat melanoma. Combination therapy can improve outcomes by focusing on several pathways implicated in the melanoma growth and progression (Munhoz & Postow, 2021).

Combination therapy has been shown to be successful in treating melanoma in several clinical trials. For instance, a phase III clinical trial found that patients with BRAF V600E/K-mutant melanoma who received the combination of dabrafenib and trametinib, two targeted therapies that target various proteins in the MAPK pathway, had significantly longer progression-free survival and overall survival than those who received dabrafenib alone (Robert et al., 2015). In a different research, individuals with BRAF V600-mutant melanoma who received both immunotherapy (ipilimumab) and targeted therapy (dabrafenib and trametinib) together saw greater response rates and longer progression-free survival than those who received either treatment alone (Larkin et al., 2015). So, the drug combination is utilized to treat melanoma because it has the ability to target numerous disease-related pathways and enhance therapeutic effectiveness. According to these research, individuals with advanced melanoma may experience better response rates, longer life times, and a delay in the emergence of drug resistance while receiving several therapies (Czarnecka et al., 2020).

1.2 Aim

The major goal of combination therapy is to create a synergistic effect, in which the individual medications work together more effectively than they would separately (Fridman et al., 2012).

1.3 Objective

Combination therapy for melanoma is significant since it has completely changed the way that this lethal and aggressive cancer is treated. For individuals with advanced melanoma, treatment outcomes have significantly improved over the past ten years as a result of the development of combination therapy. These treatments have been demonstrated to improve overall survival, boost response rates, and slow the spread of the disease. Additionally, medication resistance, a significant obstacle in the treatment of melanoma, may be addressed through combination therapy (Luke et al., 2017)

Chapter 2

Classification and Staging system of Melanoma

Melanoma is classified into four major subtypes: type I (superficial spreading), type II (nodules), type III (lentigo maligna), and type IV (acral lentiginous) (Akbani et al., 2015). The superficial spreading type continues to be the most prevalent of these and accounts for over 70% of melanomas, followed by the nodular form, which accounts for 15% to 30% of melanoma occurrences. . whereas, Acral lentiginous melanoma is a rare kind that can develop on the palms of the hands, soles of the feet, or under the nails, Less than 10% of cases of melanoma are in the lentigo, maligna and acral lentiginous types (Liu & Sheikh, 2014). On the other hand, the procedure of staging identifies the location and amount of a cancer's spread within a person's body. Stages of cancer are numbered 0 through IV. With stage IV cancer similar to a cancer that has metastasized to other distant sites. The widely used TNM (Tumor, Node, and Metastasis) staging technique is used to stage solid tumors, including melanoma (Papageorgiou et al., 2021). In terms of staging, four staging systems are used namely, the Breslow scale, the TNM staging system, the Clark staging system, and the Number staging system. The Clark scale measures how deeply a lesion has affected different skin layers whereas, Melanomas located deeper than the skin's surface are measured using the Breslow scale (Liu & Sheikh, 2014). TNM (Tumor, Node, Metastases) staging is also used for clinical staging and is based on the thickness of the lesion and an assessment of how far it has advanced to lymph nodes and other bodily tissues, as recommended by the American Joint Committee on Cancer (AJCC) (Balch et al., 2000). Stage 0 skin cancer, for example, is still confined to the epidermis (in situ) and hasn't spread to the dermis or any other deeper layers of skin. In contrast, metastases to distant organs such the lung, liver, or brain characterize Stage 4 (Balch et al., 2000).

2.1 Diagnosis

A clinician's unaided eye examination of the skin lesion can be used to diagnose melanoma. The "ABCDE rule," which is intended to identify A: asymmetry, B: uneven border, C: colour variations, D: diameter >6 mm, and E: elevated surface, is commonly used by clinicians to evaluate lesions (Akbani et al., 2015; Sboner et al., 2003). However, as indicated by the approximately 80% accurate diagnosis rate among dermatologists and the approximately 30% rate for non-dermatological professionals, diagnosis made with the unaided eye is not always reliable (Liu & Sheikh, 2014). A dermoscope or skin surface microscope enhances the visibility of the lesion. The advancement of today's digital technology has allowed for the creation of an automated melanoma diagnosis system (MEDS). In order to produce correct diagnoses, MEDS combines a number of classification algorithms and applies them to different patient lesion measurements and attributes (Sboner et al., 2003).

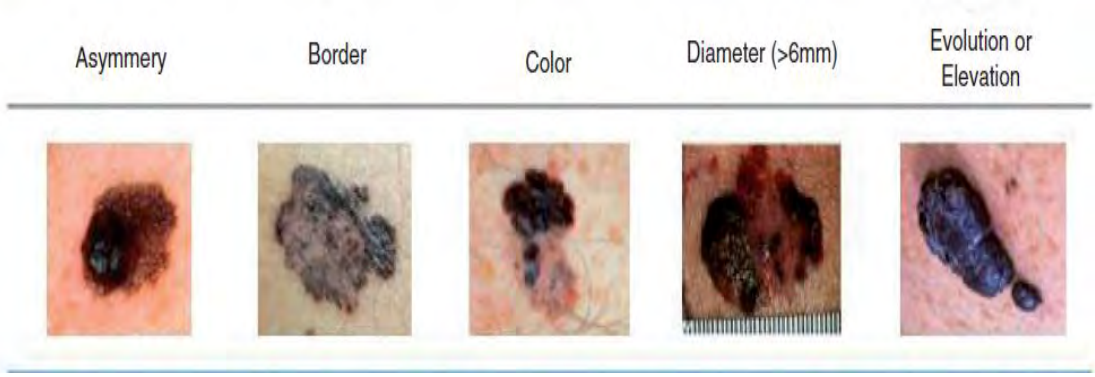

Figure 1*: ABCDE System for Diagnosis of Melanoma* (Deschenes, 1987).

The advancement of today's digital technology has allowed for the creation of an automated melanoma diagnosis system (MEDS). In order to produce correct diagnoses, MEDS combines a number of classification algorithms and applies them to different patient lesion measurements and attributes (Sboner et al., 2003). Investigations into melanoma diagnosis have also focused on discovering disease-specific biochemical markers that might be used for prognosis. For

example, the examination of cancer-free melanoma patients' blood samples for the presence of cancer cells and other mRNA (Hoon et al., 2000). Another potential diagnostic molecular marker is the microphthalmia transcription factor (Mitf). Melanoma melanocytes have been shown to express Mitf solely. The Mitf antibody may also be able to detect and stain melanocytic lesions that the more common markers HMB-45 and S-100 have missed (Liu & Sheikh, 2014) .

2.2 Aetiology and Pathogenesis

Melanocytes, the cells responsible for creating melanin in the skin, are the origin of melanoma. The genesis and pathophysiology of melanoma include interactions between genetics, the environment, and individual behavior.

2.2.1 Ultraviolet (UV) Radiation

The most significant risk factor for developing Cutaneous Melanoma (cM) is exposure to ultraviolet (UV) radiation (Kozmin et al., 2005). UV rays can originate from both natural sunlight and man-made sources. Electromagnetic waves having a length of between 200 and 400 nm can be found in the light spectrum of this radiation. The wavelength range from 290 to 320 nm is the most damaging to the skin (Carr et al., 2020). The nuclear proteins and acids of skin cells, especially melanocytes, absorb the vast majority of this radiation. Oxidative stress, which interferes with melanocyte activity, is a direct connection. Indirectly, these rays can harm cells by interfering with DNA repair procedures. This permanent damage promotes many mutations, leading to carcinogenesis and the transformation of healthy cells into malignant ones (Strashilov & Yordanov, 2021).

2.2.2 Skin Pigmentation Types

Many different skin phototypes have been observed, however they may be sorted into one of

six categories (I–IV). Fair, blond, or red hair is characteristic of people with skin phototypes I and II. People who have fair skin, a lot of freckles, or blue eyes are especially susceptible to sun damage. Therefore, these individuals have a higher chance of developing skin melanoma since they have a lower UVB radiation tolerance (Trakatelli et al., 2017).

2.2.3 Pigmented Nevi

Pigmented nevi are benign skin growths caused by melanocytes. Most of the time, pigmented nevi do not grow or behave differently during the course of an individual's lifespan. Nearly one-third of all melanomas start out as pigmented nevi. Those who have a number of different colored or shaped nevi on their skin include greater susceptibility to contracting cM. It has also been shown that they tend to have skin phototypes I and II (Strashilov & Yordanov, 2021).

2.2.4 Geographical Specifics

The prevalence of cM varies geographically. The global highest prevalence is seen in Oceania, specifically Australia and New Zealand. Northern Europe, especially Scandinavia, is home to the continent's highest concentration of this phenomenon. Eastern and Southern Europe have the lowest rates of cM (Strashilov & Yordanov, 2021).

2.2.5 Genetic Factors

cM is the result of a combination of several different genetic abnormalities in the melanocytes. DNA damage often causes these proteins to activate cell growth and inhibit apoptosis (Dahl & Guldberg, 2007). The primary cause of inherited differences is this approach. Between 15 and 20% of cases (Muñoz-Couselo et al., 2017) include NRAS as the initial gene to be altered in a very precise way. Nodular and thicker (>1 mm) melanomas, which are melanomas caused by prolonged exposure to the sun, are where the mutation is most commonly seen (Cui et al., 2007). Mutations in the BRAF gene are another common cause of cM. It's involved in around 50% of the instances. BRAF kinase controls cell proliferation and differentiation via

modulating the MAP/ERK signaling pathway. Melanoma develops when melanocytes proliferate uncontrollably (Strashilov & Yordanov, 2021) .This mutation is strongly linked to the progression of the disease, and it is highly prevalent in dysplastic nevi. In addition, UV-exposed melanoma cells have this feature. Thirdly, alterations in the gene PTEN, which encodes a tumour suppressor protein, may have a role in the aetiology of melanoma. When a tumour suppressor protein is destroyed, it loses its ability to prevent the unchecked growth of cancer cells. Ten percent to twenty percent of those with initial melanoma carried this mutation. (Strashilov & Yordanov, 2021)

2.2.6 Heredity

Gene mutations that run in families include those in CDKN2A, CDK4, POT1, ACD, TERF2IP, TERT, BRCA 1 and 2, MITF, TP53, XPC, XPD, XPA, and PTEN (Abdo et al., 2020) . Problems with cell division control have been linked to damage to the CDKN2A gene on chromosome 9. CDKN2A mutations are the most common cause of hereditary melanoma. Skin cancer runs in 20% of families with a history of it (Soura et al., 2016). The chromosome 12 CDK4 gene controls cell division. Single-cM incidence of mutations in this gene are common. Skinny melanoma is caused by CDKN2A P14 deletion mutations, which also play a role in pancreatic cancer. Because of this, we now understand why these two neoplasms tend to develop in certain families (Abdo et al., 2020; Strashilov & Yordanov, 2021). Another cause is TP53 mutations, which occur in the somatic gene that codes for p53. In its normal form, p53 acts as a tumor suppressor by facilitating the repair of damaged cells and slowing the progression of cancer. The protein produced from a defective gene is non-functional and has no effect in the body. (Soura et al., 2016).

2.2.7 Immunosuppressive Conditions

Cancer-suppressor genes like p53 and PTCH are turned off by the systemic

immunosuppression of cyclosporine and sirolimus used in kidney transplantation, whereas proto-oncogenes like H-ras, K-ras, and N-ras are turned on. All of this keeps DNA around and damages it. Skin cancer, or melanoma, develops and metastasizes due to a mutation in the genetic code (Kearney et al., 2017). Long-term immunosuppression does not successfully protect the body from acquiring and growing a range of solid tumours, including skin melanoma, making it more likely in individuals with immunosuppressive conditions like AIDS (Kubica & Brewer, 2012).

2.2.8 Melanoma-Free Skin Cancer

Melanoma skin cancer has several potential precursors including squamous cell carcinoma, basal cell carcinoma, and actinic keratosis. Both hereditary susceptibility and chronic solar exposure are likely to blame. (Strashilov & Yordanov, 2021).

2.3 Melanoma Pathogenesis

Melanomas have complex pathogenesis that include both genetic and environmental factors. The transition from the G1 to the S phase of the cell cycle is regulated by environmental factors that serve as a CDK inhibitor, preventing the phosphorylation and consequent deactivation of the retinoblastoma protein (Rb protein). In contrast, p14ARF prevents the inactivation of the tumor suppressor p53, hence reducing cell growth. When p53 is produced, the cell cycle stops in the G2/M phase or apoptosis begins (for example, in reaction to DNA damage induced by UV radiation) (Lugović-Mihić et al., 2019).

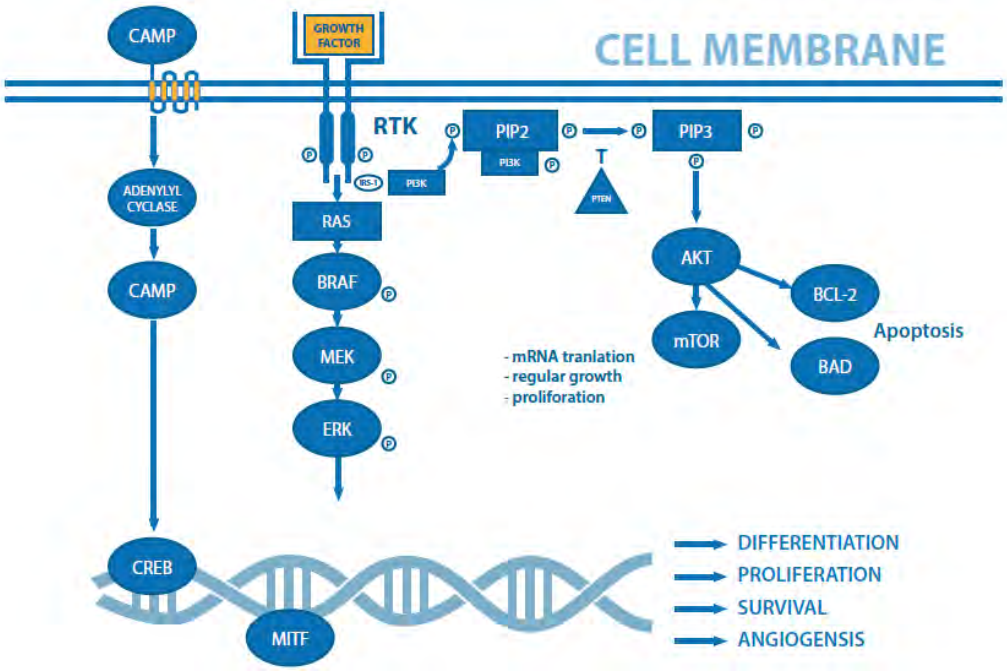

***Figure 2**: Crucial pathways in melanoma pathogenesis* (Lugović-Mihić et al., 2019).

2.3.1 MAPK Signaling Cascade

Proliferation, growth, and migration are all under the control of the MAPK signal pathway. The vast majority of melanomas, over time, melanoma causes an abnormal activation of the mitogen-activated protein kinase (MAPK) pathway. (Lugović-Mihić et al., 2019). When a growth factor binds to a receptor tyrosine kinase, a cascade of kinases is set in motion. In addition to the RAS family and monomeric G proteins, serine/threonine kinases and ERK (also known as MAPK) are activated upon receptor activation. The serine/threonine kinase ERK stimulates cellular proliferation, survival, and migration by activating transcription factors (Leonardi et al., 2018). This approach is critical since NRAS or BRAF mutations are present in 80% of cutaneous melanomas and melanocytic nevi. Most melanomas include mutations in the MAPK pathway, specifically in the BRAF serine/threonine kinase (40-50%) and the G protein NRAS (15-20%) (Lugović-Mihić et al., 2019).

2.3.2 BRAF Pathway

RAS directly turns on BRAF, which is a serine/threonine kinase that is found in large amounts in melanocytes, brain tissue, sperm, and blood cells. BRAF stimulates and phosphorylates MEK, a signalling pathway component and kinase, to enhance melanomas' proliferation and transformation (Leonardi et al., 2018). 70% of BRAF mutations are thymidine to adenine conversions (T A) that substitute valine for glutamate (V600E) and activate the kinase domain constitutively (Liu & Saeed Sheikh, n.d.). These mutations break BRAF's intramolecular connections, activating it indirectly. Melanoma with a BRAF (V600E) mutation is more common among occasional sunbathers than acral or mucosal melanoma. V600K mutations, which account for 20% of BRAF mutations in melanoma, are an example of potential replacements. BRAF V600 mutations, seen in 80% of benign and dysplastic nevi, constitute an early stage in melanoma development (TCGA, 2015). BRAF (V600E) mutation-induced nevus formation may be caused by p16 ink4A accumulation, cellular proliferation, and oncogene-induced ageing. Thus, 10% of radial-phase and 6% of in situ melanomas had mutant BRAF. Polyclonal primary melanomas and nevi contain BRAF wild-type and mutant cells. Metastatic melanomas lack polyclonality (Leonardi et al., 2018) . Females had higher BRAF V600E mutations and they decreased with age. The most prevalent mutation, BRAF V600K, rose with age (TCGA, 2015).

2.3.3 RAS Pathway

Melanoma cells proliferate less often than other solid cancers due to RAS mutations. Somatic mutations in NRAS genes (which cannot be "excluded") activate the NRAS protein's constitutive activity, which activates serine/threonine kinases in a cascade that promotes cell cycle progression, transformation, and survival (Liu & Saeed Sheikh, n.d.). Overexpression and hyperactivation of growth factor receptors like c-Met, EGFR, and KIT, and loss of function of the neurofibromatosis type 1 (NF1) tumour suppressor gene, which inhibits NRAS signaling,

may also contribute to this cascade (Lugović-Mihić et al., 2019). Only 10-20% of melanomas (mainly amelanotic nodular subtypes) have activating RAS mutations, predominantly NRAS (Kiuru & Busam, 2017). NRAS-activating mutations activate both pathways, but BRAF mutations exclusively activate MAPK (Kiuru & Busam, 2017). Since NRAS and BRAF mutations seldom occur simultaneously, only one is needed for constitutive MAPK pathway activation (Lugović-Mihić et al., 2019).Activating BRAF mutations outnumber NRAS mutations in 70–80% of dysplastic nevi. HRAS mutations are connected to Spitz nevi. Due to hyperexpression or activation of growth factor receptors such c-Met, KIT, and EGFR, melanomas may dysregulate the AMPK signaling pathway (Lugović-Mihić et al., 2019).

2.3.4 C-KIT Pathway

According to a study melanocyte maturation requires stem cell factor and c-KIT (Leonardi et al., 2018). Therefore, insufficient pigmentation results from c-KIT mutations. Numerous immunohistochemical studies have linked the lowering of c-KIT expression to the development of primary or metastatic melanoma. Sun-exposed cutaneous melanomas and acral melanomas (hands, foot, and nail bed) revealed activating alterations and KIT gene amplifications (Davies, 2018).

In addition to the PI3K-AKT pathway, KIT mutations can activate other signaling pathways. The KIT gene alterations shown by the dots are identical to those observed in GIST. Since the clinical response rate to KIT inhibitors in melanomas is much lower (10-30%) than in GIST (> 70%), the functional characteristic of these mutations turned out to be therapeutically significant (Davies, 2018).

2.3.5 C-MET and HGF

Overexpression of the tyrosine kinase receptor c-MET and its ligand, hepatocyte growth factor, is linked to the development of melanomas (Lugović-Mihić et al., 2019). Tumors arise and

cancer cells metastasize when their dysregulation is caused by inappropriate c-MET activation; this is because c-MET governs a wide variety of biological activities, including proliferation, survival, motility, and invasion. It is important to remember that the melanoma tumor microenvironment can cause an overactive form of the tyrosine kinase receptor c-MET. Tumour cells' PI3K-AKT pathway is activated by this paracrine activity, which also increases their resistance to MAPK inhibitors (Davies, 2018).

2.3.6 PI3K/PTEN/AKT Pathway

Cell signaling depends on PI3K-AKT. It regulates cell growth, death, and survival. Activating mutations (like AKT1) or removing functional pathway components like PTEN and PIK3CA (Schadendorf et al., 2015). RAS activation of tyrosine kinase receptors may activate PI3 kinases directly or indirectly. They phosphorylate PIP2 into PIP3, which phosphorylates AKT (Schadendorf et al., 2015). PTEN lipid phosphatase, which converts PIP3 to PIP2, is a key antagonist of this system. AKT phosphorylation affects cell proliferation, survival, motility, angiogenesis, and metabolism via multi-factor proteins. Seventy-three percent of human melanoma cell lines, but few nevus cells, activate m-TOR. Many melanomas have increased PI3K signaling due to PTEN inhibitor gene mutations, deletions, and promoter methylation (Strashilov & Yordanov, 2021).

2.3.7 MITF Transmission Protocol

Microphthalmia-associated transcription factor (MITF) controls melanocyte development and may alter the malignancy of certain melanomas. 15–20% of melanomas, notably metastatic ones, have MITF gene amplification (Ballesteros-Álvarez et al., 2020). These alterations may occur later in the disease and lower 5-year survival. MITF's cell cycle participation upregulates these genes. P16INK4a and p21 transcriptionally control BCL-2, an anti-apoptotic mitochondrial membrane protein (Strashilov & Yordanov, 2021). Melanocortins (ACTH and -

MSH) activate the MC1R to produce MITF. Thus, adenylate cyclase activates PKA to produce c-AMP. CREB, a transcription factor, increases MITF production after PKA activation. MAPKs control MITF signaling. BRAF controls MITF production to optimize melanoma cell proliferation and survival. MITF may also be a transcription factor that inhibits cell division. Genes controlling melanocyte growth, pigmentation, distribution, and survival are MITF targets upon melanocyte-stimulating hormone (MSH) binding to the melanocortin 1 receptor (MC1R) (Ballesteros-Álvarez et al., 2020).

2.3.8 The NF1 Gene's Signal Transmission

Neurofibromin, sometimes called NF-1 or neurofibromatosis-related protein, is encoded by the NF1 gene. It is a GTPase-activating protein. Neurofibromin controls cell growth. Ability to adapt and specialize in order to survive. As the first protein in the mitogen-activated protein kinase (MAPK) signal transduction cascade, neurofibromin acts as a negative regulator of RAS by catalysing the hydrolysis of RAS-GTP to RAS-GDP. Loss of neurofibromin 1 (NF1) causes an increase in melanin production in melanocytes (Czarnecka et al., 2020). In the absence of neurofibromin, several signaling pathways are enhanced, promoting cell growth and survival.

2.3.9 The p53 Signal Transmission

p53, the main tumour suppressor gene, causes programmed cell death. This gene produces the transcription factor p53, which reacts to DNA damage, genomic instability, hypoxia, and neoplastic abnormalities (Strashilov & Yordanov, 2021). Melanoma cells have uncontrolled proliferation, genetic instability, and cell cycle issues. 50% of carcinomas had mutant or deleted p53. 11-25% of metastatic melanomas have them, whereas just 1%-5% of original ones (Strashilov & Yordanov, 2021). Melanomas seldom express P53 protein, unlike nevi. Melanoma overexpresses this protein more than other cancers. Melanoma cells defy programmed cell death despite strong p53 expression. Due to p53 apoptotic pathway

dysfunction (Strashilov & Yordanov, 2021).

2.3.10 Crucial to Melanoma Pathogenesis: Other Factors

Telomerase reverse transcriptase (TERT) on chromosome 5p15.33 encodes the catalytic component. Nucleotides are added to telomeres by telomerase. The lack of its action in healthy cells leads to their premature ageing and eventual demise. The TERT gene's promoter mutations have been detected in 77% of melanoma precursor lesions and a large percentage of melanoma cells. This leads to increased telomerase activity, which in turn slows down the ageing and death of cells. Melanomas with TERT gene mutations have a dismal prognosis (Shain et al., 2015).

Melanoma's aetiology is also affected by cellular interactions mediated by adherents and c-adherins, cell adhesion molecules. The immune system is critically important in the development of melanoma. It takes both humoral and cell-mediated immune responses to effectively combat melanoma. Impaired antigen presentation, immunological barriers in the tumor microenvironment, negative regulatory pathways in T cells, and defective T cells are only some of the ways in which cancer cells can exert control over the immune response (Konsoulova, 2015)

To keep the immune system in check, PD1 and CTLA-4 suppress T cell activation. This disease's therapy has been revolutionized by the eradication of CTLA-4 (ipilimumab) and PD1 (nivolumab, pembrolizumab) reduction of T cell activity and the restoration of T cell identification ability. Using these antibodies improves treatment response and overall survival (Konsoulova, 2015). The majority of advanced and metastatic melanoma patients are treated with these advances, which are also used to treat other solid tumours and haematological malignancies (Weiss et al., 2019).

Malignant neoplasms can spread to other parts of the body. Cell-cell adhesion molecules such

as E-cadherin link melanocytes to basal keratinocytes. Melanoma cells become detached from the epidermis and invasive when they lose E-cadherin and overexpress N-cadherin. The cell adhesion process has been hypothesized to be regulated by a number of proteins, including phosphatase and tensin homologue (PTEN). (Strashilov & Yordanov, 2021) .

2.4 Treatment Strategies

From cytotoxic chemotherapies like dacarbazine to targeted therapies and immunotherapies like immune checkpoint inhibitors, melanoma treatment has come a long way in the past decade. In general, the advent of these pharmaceutical advances has extending the time that melanoma patients with advanced disease can live without dying from the disease. In order to overcome the difficulties presented by monotherapy resistance, modern methods make use of combination drugs that make use of synergy across anticancer effectiveness mechanisms (Chanda & Cohen, 2021).

Chapter 3

Mechanisms of Action for Melanoma Therapies: Combinatorial Approaches

3.1 Cytotoxic Chemotherapy Combinations

For a long time, cytotoxic chemotherapy was the go-to treatment for metastatic melanoma. However, as additional options have become available, researchers have shifted their focus away from developing new cytotoxics. For melanoma, dacarbazine has been the only cytotoxic chemotherapy since it was approved by the FDA in 1974 (Chanda & Cohen, 2021). Many medications were once thought to improve the efficacy of combination chemotherapies when added to dacarbazine or temozolomide (Coit et al., 2019). High response rates of up to 55% were achieved with the Dartmouth regimen (CBDT: cisplatin, carmustine, dacarbazine, and tamoxifen), and complete responses were maintained for up to 82 months (Smalley et al., 2016a). In addition to cisplatin, vinblastine, and dacarbazine, the BOLD (bleomycin, vincristine, lomustine, dacarbazine) and CVD (cisplatin, vinblastine, dacarbazine) chemotherapy regimens are also effective against metastatic melanoma (Chanda & Cohen, 2021). However, none of these combinations were more effective than dacarbazine alone. These were ultimately abandoned due to their inferiority to dacarbazine alone and the higher toxicity observed when other therapies were taken. As more selective targeted medications and immunotherapies have become available, cytotoxic chemotherapy is now considered a second-line therapy for patients with metastatic melanoma (Coit et al., 2019).

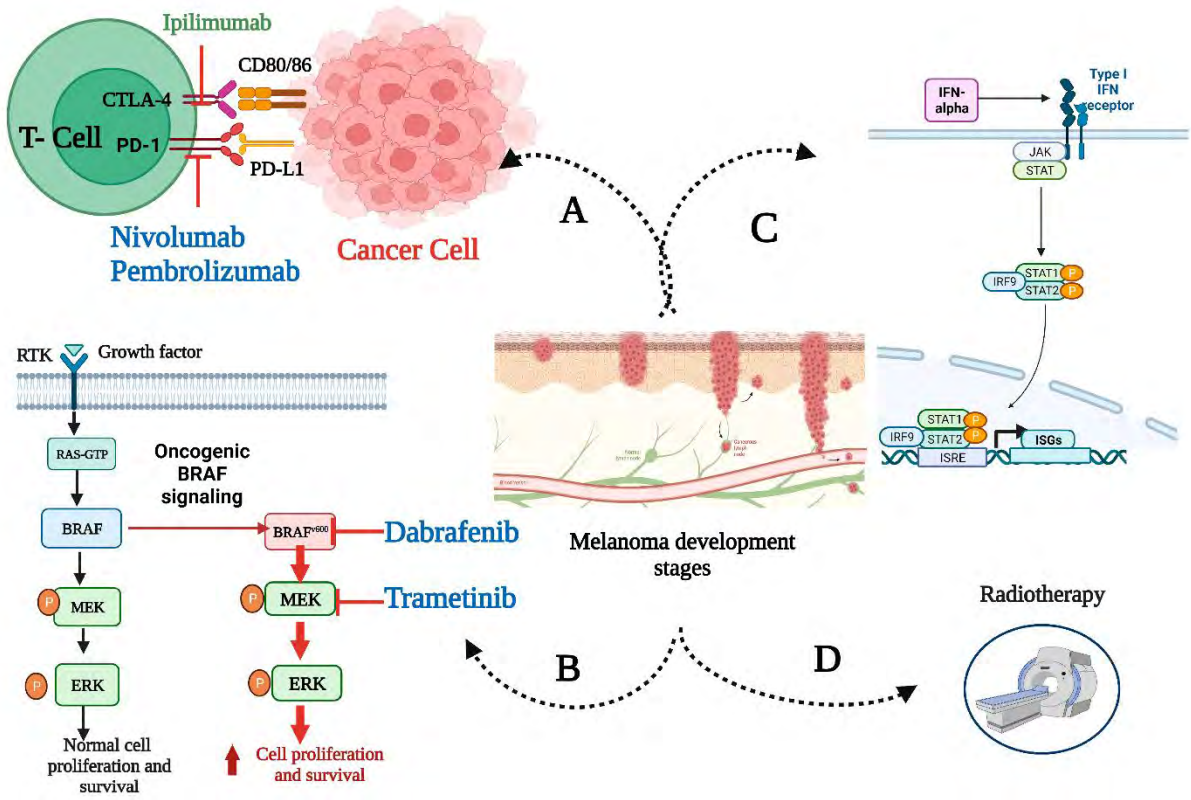

***Figure 3**: Approved Mechanisms of combinatorial therapies for high-risk melanoma* (Eljilany et al., 2023).

3.2 Immune Checkpoint Blockade Combinations (Nivolumab and Ipilimumab)

Combination immunotherapy with nivolumab and ipilimumab for advanced (stage IV or unresectable stage III) melanoma was approved by the FDA following evaluation in phase 1, 2, and 3 trials (Spain & Larkin, 2016). Both nivolumab and ipilimumab, by inhibiting separate checkpoints, improve the immune response. With this strategy, drugs prime the immune system to attack melanoma cells and penetrate tumours (Krattinger et al., 2021). Ipilimumab inhibits CTLA-4, a molecule involved in immune system regulation. T cells are a kind of white blood cell that eliminates foreign cells, such as cancer cells, from the body. The expression and function of CTLA-4 in T cells is regulated. The anti-CTLA-4 antibody ipilimumab enhances the immune response against melanoma by stimulating the development of T cells (Larkin et

al., 2019). Nivolumab prevents the immune system from attacking cancer cells by blocking a different checkpoint protein called PD-1. Nivolumab removes the "shield" provided by PD-1, making it possible for the immune system to detect and kill melanoma cells (Spain & Larkin, 2016). Ipilimumab stimulates T cells to target melanoma. Nivolumab eliminates the immunological defense that cancer cells have developed to ward off danger. Systemic drugs, such as nivolumab and ipilimumab, are distributed to all parts of the body via the circulation. Oncologists utilize systemic immunotherapy to treat metastatic cancer, which has spread beyond its original location (Spain & Larkin, 2016).

3.3 MEK + BRAF Inhibitor Combinations

The molecules BRAF and MEK play critical roles in regulating cell growth. A small number of BRAF mutations lead to uncontrolled cell division and abnormal growth. It is possible for a melanoma tumour to form from these cells. About half of all melanomas are caused by a BRAF mutation. MEK receives signals from BRAF and other cellular components. Scientists found that combining BRAF and MEK inhibition yields better results than MEK inhibition alone (Subbiah et al., 2020). The multikinase inhibitor sorafenib was initially utilized in attempts to directly target abnormal MAPK pathway signaling in patients with melanoma who had oncogenic BRAF mutations. Sorafenib performed poorly (Robert et al., 2015). Weak affinity for mutant BRAF at therapeutically practical concentrations, which may explain its lack of clinical efficacy in melanoma patients, alone or with treatment. Selective BRAF inhibitors (BRAFi) like Targeted therapy vemurafenib, dabrafenib, and encorafenib performed well (Long et al., 2015). BRAFi was challenged within months. MEK inhibitor combination therapy enhanced outcomes and was FDA-approved for specific combinations. BRAF V600 mutations in other malignancies were promising. Wild-type cells activate the MAPK pathway, which promotes resistance and makes it hard to regulate cutaneous events (Subbiah et al., 2020).

Four randomized, phase 3 studies confirmed that concurrent administration of BRAF inhibitors

(vemurafenib, dabrafenib, or encorafenib) and MEK inhibitors (cobimetinib, trametinib, or binimetinib) was superior to concurrent administration of single-agent BRAF inhibitors in terms of ORR, PFS, and OS. This led to the FDA's approval of three of these drugs. Encorafenib+ Binimetinib , Dabrafenib + Trametinib, and Vemurafenib + Cobimetinib (Subbiah et al., 2020)

3.4 Triplet Therapy

Targeted therapies are currently used to treat advanced BRAF mutant melanoma. Antibodies against PD-1, PD-L1, and CTLA-4 are examples of CPIs; BRAF/MEKi are also part of this class. Triplet melanoma treatment utilizes a combination of three medications that destroy cancer cells in a variety of ways (Dixon-Douglas et al., 2022).

Mutations in the mitogen-activated protein kinase (MAPK) pathway are a primary cause of melanoma, and MEK and BRAF inhibitors work by blocking this route. Inhibiting these pathways slows the rapid growth and division of cancer cells. Immune checkpoint inhibitors, such as PD-1 and CTLA-4 inhibitors, boost the body's immune response, making it more effective in recognising and killing cancer cells (Garzón-Orjuela et al., 2020). They free T cells, allowing them to initiate an immune assault on melanoma cells that is stronger and lasts longer. It is possible that the use of targeted therapies in conjunction with immunotherapies will have synergistic effects, making the use of both medicines more successful than using either alone. By simultaneously targeting many aspects of cancer cells, therapeutic efficacy is increased (Weiss et al., 2019). Clinical trials using triplet combinations to treat melanoma have shown promising results, increasing response rates and survival for certain patients (Dixon-Douglas et al., 2022). However, not everyone may be a suitable fit for this method, and it is crucial to remember that treatment plans are extremely personalized. The optimum course of action is determined by a number of factors, including the patient's overall health, the genetic composition of the tumour, and any previous treatment experiences. This decision, like that of

any cancer treatment, is best made in consultation with a melanoma treatment team or oncologist (Dixon-Douglas et al., 2022).

3.5 Radiation and Immune Checkpoint Inhibitors Combination

Radiation therapy (RT) raises PD-L1 expression on cancer cells. Blocking PDL1/PD-1 signalling with antibodies helps CD8 T cells recover their function following RT stimulation. These fully operational CD8 T cells may effectively target and kill cancer cells, leading to tumour necrosis and an inflammatory response (Voronova et al., 2022).

3.6 Chemotherapy and Immune Checkpoint Inhibitors Combination

As a consequence of chemotherapy, cancer cells might die, unleashing a variety of tumour antigens (proteins) into the tumour microenvironment. Increased antigen presentation may occur if antigen-presenting cells (APCs) take up cancer antigens and display them. After being activated, these APCs can help T cells, a key immune system component, target and destroy cancer cells (Krattinger et al., 2021). Chemotherapy may induce an inflammatory response in the cancer microenvironment. The capacity of this inflammation to attract immune cells may make the tumour more susceptible to immunological attack (Marconcini et al., 2022). There is a synergy with ICIs because of their capacity to block immunological checkpoints, which keeps T cells targeting cancer cells. When chemotherapy is used in conjunction with ICIs, the immune response to cancer cells is bolstered by the enhanced antigen presentation and inflammatory response (Krattinger et al., 2021; Marconcini et al., 2022). Reducing Immunosuppressive Cells in the Tumour Microenvironment to Overcome Immune Suppression: Some chemotherapy medications, especially immune-modulating chemotherapy, can lower immunosuppressive cells to help overcome the inhibitory signals that prevent the immune system from effectively attacking cancer cells (Krattinger et al., 2021).

Chapter 4

Efficacy Data of Combinatorial Therapies of Melanoma

Combinatorial approaches to treating metastatic melanoma are shown in the table, which indicates the efficacy of presently authorized immuno-checkpoint blocker and combined BRAF and MEK inhibitor combinations. These results suggest that combinatorial approaches may increase anticancer effectiveness while decreasing toxicity in some settings. Combinations of BRAF/MEK inhibitors outperformed single-agent BRAF inhibitors without increasing the absolute incidence of side events. The importance of weighing the extent of benefit when faced with several treatment options is shown in the table.

The reactivation of the MAPK pathway at a rate of 70% limits the effectiveness of single-agent BRAF inhibitors. Combining BRAF inhibitors with MEK inhibitors delays treatment resistance and boosts their anticancer effects (Subbiah et al., 2020). Combination therapy with BRAF and MEK inhibitors has been shown to be superior to either therapy alone in four randomised phase 3 studies assessing ORR, PFS, and OS. Overall response rates (ORRs) of 70% and complete response rates (19%) were achieved when dabrafenib and trametinib were administered simultaneously (Zhou & Johnson, 2018). Patients with normal LDH levels and fewer metastatic locales had better responses and survival rates (Subbiah et al., 2020). The coBRIM study found that as compared to vemurafenib + placebo, OS was significantly better when combination blocking was used (Smalley et al., 2016b). Overall response rate (ORR) of 64% and median progression-free survival (PFS) of 14.9 months were beneficial for the combination of encorafenib and binimetinib (Subbiah et al., 2020).

The combination of ipilimumab and nivolumab showed a 40% objective response rate (ORR) and 53% treatment-related adverse events (AEs) in a phase 1 trial for patients with advanced melanoma (Larkin et al., 2019). The combination of nivolumab 1 mg/kg and ipilimumab 3

mg/kg was selected for phase 2 and 3 studies because of its efficacy and demonstrated overall survival (OS) benefit (Larkin et al., 2019). Following FDA approval of the CheckMate-069 and CheckMate-067 investigations, patients with metastatic melanoma were given a combination of ipilimumab and nivolumab. The overall response rates for both studies were 61% and 58%, and 54% and 59% of patients experienced adverse events of grade 3 or 4 (Munhoz & Postow, 2021; Spain & Larkin, 2016)

Table 1: Efficacy Data of combinatorial therapies of melanoma (Munhoz & Postow, 2021; Spain & Larkin, 2016; Subbiah et al., 2020).

Combination therapies of melanoma	**Targeted pathways**	**Efficacy Data**			**Remarks**
		Objective response Rate (ORR) %	Overall Survival (OS)	Progression free Survival (PFS)	
Dabrafenib + trametinib (150mg+2mg)	BRAF+MEK	68%	25.9 months	11.2 months	The CTLA-4 + PD-1 pathway inhibitor combination "nivolumab + ipilimumab" (3mg/kg + 1 mg/ml) was shown to be more effective than other existing CTLA-4 + PD-1 inhibitor combinations in the treatment of melanoma. Encorafenib and binimetinib (300 mg + 45 mg), which block
Vemurafenib + cobimetinib (1920mg+60mg)	BRAF+MEK	70%	22.5 months	12.3 months	
Encorafenib + binimetinib (300mg+45mg)	BRAF+MEK	64%	33.6 months	14.9 months	
Nivolumab + Ipilimumab (3mg/kg+1mg/ml)	PD-1 + CTLA-4	58%	~ 6 years	11.5 months	
Pembrolizumab + Ipilimubab (2mg+1mg)	PD-1+ CTLA-4	30%	10.9 months	4.1 months	
Dabrafenib + Trametinib + Pembrolizumab	BRAF/MEK + PD-1	63.3%	NR	16.9 months	
Vemurafenib + Cobimetinib + Atezolizumab	BRAF/MEK + PD-L1	66.3 %	NR	15.1 months	
Dabrafenib + Trametinib + Spartalizumab	BRAF/MEK + PD-L1	68.5%	NR	16.2 months	
Radiotherapy+Ipilimumab	CD8 T cell + CTLA-4	37.1%	19 months	5 months	

Dacarbazine +Ipilimumab (10mg/kg + (850mg/m2)	APCs +CTLA-4	15.2%	11.2 months	NR	BRAF/MEK, are more efficacious than other BRAF/MEK inhibitors. These effectiveness statistics from melanoma clinical trials are provided in terms of the ORR (Overall Response Rate), OS (Overall Survival), and PFS (Progression-Free Survival).

Combination immune-checkpoint blockers (ICB) have been associated with a high rate of immune-related AEs (Long et al., 2017). The therapy of inhibiting ICBs for metastatic melanoma has been plagued by these effects. It has been a goal to find different ways to lessen these toxicity levels. In the phase 1b KEYNOTE-029 research, 153 patients with advanced melanoma were treated with a combination of "low dose" pembrolizumab (2 mg/kg) and "standard dose" ipilimumab (1 mg/kg) (Marconcini et al., 2022). Pembrolizumab alone was supplied for up to two years following the initial treatment phase of four doses every three weeks (Munhoz & Postow, 2021). The ORR in this study was 57%, and 12 months later, 89%

of patients were still alive. Grade 3-4 AEs occurred in 45% of cases, while only 14% of patients stopped treatment because of them (Munhoz & Postow, 2021). Although this study was not randomized, it was the first to suggest that modest doses of ipilimumab coupled with anti-PD1 could be as effective as higher doses of ipilimumab alone while causing fewer adverse effects (Marconcini et al., 2022; Munhoz & Postow, 2021).

PFS in the triplet arms of all three trials, KEYNOTE-022 (dabrafenib + trametinib + pembrolizumab), IMspire150 (vemurafenib + cobimetinib + atezolizumab), and COMBI-i (dabrafenib + trametinib + spartalizumab), varied from 15.1 to 16.9 months. The IMspire150 study was the only one to show a significant improvement in PFS (Dixon-Douglas et al., 2022). While KEYNOTE-022 did not reach statistical significance in the planned study, it did show promise, suggesting that more investigation is required. The ORR in the pembrolizumab arm of KEYNOTE-022 was 63%, which was lower than the ORR in the control arm of 72% in the other two trials (Voronova et al., 2022). There was a general trend towards enhanced OS across all three tests, however these results are still preliminary. finally, The median OS in the triplet arm of IMspire150, COMBI-i, and KEYNOTE-022 was not reached, while it was 26.3 months in the placebo arm of KEYNOTE-022 (Dixon-Douglas et al., 2022).

Radiotherapy and chemotherapy have been related to immunological-potentiating processes such as the release of tumour antigens for immunological presentation, the removal of immune suppressive cells, and the activation of immune effectors in the cancer microenvironment (Hayashi & Nakagawa, 2020). An examination of past data has shown that those who get the addition of radiation to 3 mg/kg of ipilimumab increased survival time to 19 months from 10 months. Higher percentages of complete responses were seen with ipilimumab-radiation treatment (25.7%). with no further harm (as opposed to 6.5% in the ipilimumab group) (Hayashi & Nakagawa, 2020). Similar results were as a little future experiment showed. The second phase of the study is still underway. Investigating how PD-1 inhibitors react to localized

radiation. Prospective studies with larger patient populations are needed to determine the efficacy of combining radiation with ICI, although for the time being, it appears to be safe (Voronova et al., 2022).

In a double-blind phase III study, 502 patients with metastatic melanoma were randomly assigned to receive either ipilimumab 10 mg/kg plus dacarbazine 850 mg/m2 or dacarbazine alone. With the combination, OS increased to 11.2 months, up from 9.1 months. While 27.5% of patients who got just dacarbazine experienced AEs, 56.3% of those who received ipilimumab also experienced AEs of grades 3-4. Anti-chemotherapy with PD-1 inhibitors in people with advanced melanoma has not been the subject of any phase II/III research (Krattinger et al., 2021).

Chapter 5

Discussion and Future Aspects

Recent pathophysiology studies on cutaneous malignancies have led to the development of effective, individualized therapies. Particularly, new therapeutic philosophies have revolutionized the management of metastatic or unresectable melanoma. Melanoma has more mutations than any other solid tumour, and cancer cells may hide from the immune system, making it challenging to treat advanced melanoma. The treatment and survival of patients with advanced melanoma have dramatically improved over the past decade as a result of the approval of several therapies, such as BRAF, CTLA4, and PD1 inhibitors, showing promising results in terms of effectiveness and safety for the management of metastatic or unresectable melanoma. It was also shown that combining targeted medicines with immunotherapies is an effective strategy for treating melanoma. T cell agonists, intravenous oncolytic viruses, vaccines, cytokines, etc. are some of the various therapy options now being studied. However, real-world data are necessary to evaluate the effectiveness and safety of these therapies in a real-world context.

For first-line treatment, the limited data suggest that BRAF/MEK combination therapy is more effective than BRAF inhibitor monotherapy while also being less toxic. Similarly, anti-PD-1 monotherapy has been shown to be more effective than anti-CTLA-4 monotherapy while also having lower toxicity (Luke, 2019).

Most worldwide clinical recommendations have adopted anti-PD1 single treatment (nivolumab or pembrolizumab) and anti-CTLA4 and anti-PD 1 (ipilimumab plus nivolumab) as the standard of care in recent years (Vázquez-Montero et al., 2023). Long-term follow-up efficacy data from phase II and III trials, particularly the toxicity burden of the anti-CTLA4 and anti-PD1combination, have raised doubt on which treatments should be used as first-line therapy

for advanced melanoma and whether they should be used in combination or as single agents. Nivolumab plus ipilimumab combination have shown significant increase in progression-free survival and objective response rate in phase III studies of patients with metastatic melanoma, with the longest median overall survival (OS) (72.1 months or ~6 years) recorded to date. This benefit has remarkably persisted throughout several years of follow-up (Vázquez-Montero et al., 2023).

Chapter 6

Conclusion

Melanoma is an extremely lethal cancer. Because of its diversity and complexity, it may be difficult to diagnose and effectively treat. New methods for diagnosing and treating diseases, as well as insight into the mechanisms by which melanomas evade the immune system, are all within our reach. Additionally, new technologies are being developed to provide more unbiased methods for Melanoma diagnostic and prognostic tools, which will improve medical outcomes. The therapy of metastatic melanoma has advanced greatly in recent years due to the development of drugs such as BRAF, CTLA4, and PD1 inhibitors. Researchers have been working on novel drugs and pharmacological combinations to provide a longer-lasting effect. It has become clear that there are multiple pathways to secondary resistance. Investigation into the mechanisms behind the success or failure of various treatments is ongoing. To better stratify patients and provide more customized treatments based on mutational and biomarker profiles, researchers are prioritizing the creation of biomarkers that may be used to predict which patients will respond and which will not. Developing a more personalized approach to treating melanoma patients can improve prognosis and reduce treatment costs. The unintended consequences of ineffective treatments for patients will also be mitigated. No patient will be prescribed ineffective medications. Combinatorial techniques are becoming the gold standard for treating patients with advanced melanoma, and more treatment options are likely to become available in the near future. However, there is a need for more clinical trials, useful biomarkers, and translational research in order to resolve the many outstanding concerns and facilitate the sensible, patient-centered, cost-efficient integration of emerging treatments.

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