Exploring Mechanisms and Promising Treatments of Malignant Melanoma

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ABSTRACT

Malignant melanoma is one of the 4 main types of skin cancer, among basal cell carcinoma, squamous cell carcinoma, and Merkel cell carcinoma, and it is the most aggressive and metastatic form. Unfortunately, advanced-stage, metastatic patients have a 5-year survival rate of a mere 5% to 19%.

The traditional first-line treatment option for localized cutaneous melanomas is a tumor and margin excision surgery; however, in advanced-stage patients, chemotherapy drugs and targeted therapies have emerged as a more effective treatment option. The rising incidence of malignant melanoma in the fair-skinned populations of North America, Europe, and Australia, along with rising resistance to immune checkpoint inhibitors (ICIs), underscores the importance of new, efficacious melanoma treatments. The novel and breakthrough development of two types of immunotherapies, T-cell receptor (TCR) engineered T-cell immunotherapy (TCR-T) and mRNA vaccines, are promising treatment options, especially for advanced-stage, metastatic patients. While these treatments are in the early stages of development, various clinical trials have resulted in positive outcomes. Lastly, this review will highlight the challenges and setbacks to immunotherapy and future directions. Immunotherapy may become the standard for melanoma treatment, however, by overcoming current barriers, only time will tell.

Introduction

Malignant melanoma (melanoma) is one of the most aggressive and treatment-resistant cancers, the deadliest form of skin cancer, responsible for 80% of all skin cancer deaths (Paluncic et al., 2016; Sandru et al., 2014). In 2023, melanoma was responsible for an estimated 7,990 deaths because it is more likely to metastasize than other forms of skin cancer (i.e., basal and squamous cell carcinoma). However, melanoma is less frequent than other forms of skin cancer because it only accounts for about 1% of all diagnosed skin cancers (Sandru et al., 2014).

Four main types of melanoma are characterized by their different appearances and origins of occurrence. Among these types, more than 50% of cases are invasive, penetrating through the top layer of skin, the epidermis, and into the skin's second layer, the dermis (American Cancer Society, 2023). The remaining cases are noninvasive and confined to the epidermis (American Cancer Society, 2023).

The first type of melanoma is superficial spreading melanoma, the most common type of melanoma. and it can arise from an existing mole or appear as a new legion, and it tends to originate in the torso, legs, and upper back. Superficial spreading melanoma appears flat or slightly raised, and it may be asymmetrical, have uneven borders, and be discolored with shades of tan, brown, black, red, or even blue and white. A second type of melanoma is lentigo maligna, which most often develops in older people. Like superficial spreading melanoma, lentigo maligna typically grows close to the skin's surface at first, and it arises from sun-damaged skin on the face, ears, arms, or upper torso. In terms of its appearance, lentigo maligna may look flat or slightly

raised, have uneven borders, and is usually blue-black in color. The third main type of melanoma is acral lentiginous melanoma, the most common type of melanoma found in people of color. It often appears under the nails, on the soles of the feet, or on the palms of the hands. This type of melanoma can appear as black or brown in color. Lastly, the fourth main type of melanoma is nodular melanoma. Nodular melanoma is the most aggressive type of melanoma, accounting for 10 to 15 percent of all cases of melanoma (American Cancer Society, 2023). In contrast, nodular melanoma grows rapidly deeper into the skin, making it invasive at an early diagnosis. Nodular melanoma is most commonly found on the torso, legs, arms, and scalp and is recognized as a blue-black bump (American Cancer Society, 2023).

The most common method of melanoma detection is a biopsy of a suspicious skin area called a lesion, and the procedure is done by taking a small tissue sample for laboratory testing (Cancer.Net, 2023). Luckily, in cases where melanoma receives an early diagnosis, patients exhibit a favorable prognosis, boasting a 5-year survival rate of 99%. Metastatic melanoma patients have a grim prognosis, however, characterized by a 5-year survival rate ranging from 5% to 19%. (Halpern et al., 2022).

This review delves into the initial transformation of melanocytes to melanoma cells, particularly induced by UV-radiation exposure to UVA and UVB, and the consequent damage to DNA. In addition, the acquisition of genetic mutations, including in the BRAF, NRAS, and NF-1 oncogenes, plays a key role in melanogenesis by dysregulating essential signaling pathways. As a result, the MAPK/ERK and PI3K/AKT signaling pathways become constitutively activated, and they carry out anti-apoptotic effects, cell proliferation, and disruptions to cell cycle control and senescence. Furthermore, this review will briefly discuss the epigenetic mechanisms of DNA methylation and histone modifications that are linked to and serve as biomarkers for tumorigenesis. Lastly, this review briefly touches on the tumor microenvironment (TME) and its interplay with the immune system, which serves as background information to novel immunotherapy treatments.

Risk Factors for Melanoma

Melanoma originates from the neural-crest-derived melanocytes, which are the pigment-producing cells that are located in various anatomic sites of the body, notably the stratum basale of the skin's epidermis, the uvea of the eyes, and the inner ear (Paluncic et al., 2016). The transformation of melanocytes to melanoma cells is a complex and multi-step process, and it is most commonly attributed to the acquisition and cloning of genetic mutations.

The two types of UV light that are proven to increase the risk of skin cancer development include UVA (wavelength 315-400 nm) and UVB (wavelength 280-315 nm). UVA and UVB can trigger DNA damage to melanocytes through UV-induced reactive oxygen species (ROS) production (Paluncic et al., 2016).

UVB is believed to be more carcinogenic than UVA because it induces the formation of cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidone photoproducts, and it is involved in the development of oxidative stress (Paluncic et al., 2016). It is also important to note that ROS production and oxidative stress can be induced by other exogenous sources besides UV light, including tobacco and smoke exposure, pollutants, heavy metals, ionizing radiation, and xenobiotics (Juan et al., 2021). These components can also induce DNA damage, leading to the development and cloning of mutations.

Malignant melanoma is most common among people with pale skin, blue eyes, and red or fair hair. Essentially, people with a pale complexion produce less of the protective pigment melanin, resulting in a higher risk of developing melanoma. The incidence of melanoma in white populations generally increases with decreasing latitude, so the highest incidence rates are in Australia, which are 10 and 20 times over the incidence rates of melanoma in other Caucasian-dominated places in Europe for women and men, respectively (World Health Organization, 2017). In contrast, naturally brown and black populations can safely tolerate relatively high levels of UV exposure without becoming sunburnt or greatly increasing their risk of developing skin cancer (World Health Organization, 2017).

Interplay of the Tumor Microenvironment

The tumor microenvironment (TME) refers to the tumor stroma, which includes fibroblasts, keratinocytes, immune cells, soluble molecules, and the extracellular matrix (ECM) (Zhou et al., 2023).   Furthermore, the interaction between the TME and its immune cells is crucial in tumor proliferation, drug resistance development, and immune evasion. The immune system of the TME encompasses cell types, including effector T-cells, cytotoxic T-cells, CD4+ effector T-cells, natural killer cells, dendritic cells, and M1-polarized macrophages. These cells are responsible for recognizing and neutralizing antigens and triggering an immune response. The interaction between the immune system and tumors affects melanoma growth, invasion, and metastasis. Notably, in metastatic melanoma, it has been observed that dysregulated CD8+ T-cells led to proliferation, cloning, and dynamic differentiation (Zhou et al., 2023).

The TME plays an active and critical role in tumorigenesis, and in particular, certain microenvironments may induce the establishment of secondary tumors or melanoma metastasis (Villanueva & Herlyn, 2008). The dynamic interaction between the TME and tumor cells is important for the growth, local invasion, and metastatic spread of tumor cells. The interplay between the TME and the host immune system is foundational to understanding the novel immunotherapy treatments this review will discuss later.

Epigenetic Mechanisms in Melanomagenesis

Epigenetics, based on behavior and environment, involves the alteration to gene expression that does not change the actual DNA sequence. These modifications play a role in melanoma development and progression. This review will briefly delve into common epigenetic modifications that serve as biomarkers for the identification of melanoma.

Primarily, hypermethylation occurs most often at CpG islands most often occurs in the promoter regions of specific genes, including the tumor suppressor genes PTEN, CDKN2A (p16), CDKN2A (p14), and RASSF1A, which have been found in 6-62%, 5-27%, 41-57%, and 15-57% of all melanomas, respectively (Santourlidis et al., 2022). DNA hypermethylation inactivates the transcription, DNA repair, apoptosis, and cell cycle regulation genes (Fath et al., 2022). Without proper regulation of cell proliferation and DNA replication mechanisms, melanocytes are at risk of uncontrolled growth and tumorigenesis.

In contrast, hypomethylation, a loss of methylation, commonly occurs at repetitive DNA elements, including long interspersed nuclear element-1 (LINE-1). Repetitive hypomethylation of this retrotransposon may result in its reactivation, which has been linked to apoptosis, DNA damage and repair, tumor progression, and stress response, all responsible for tumorigenesis. In fact, in 75% of 16 melanoma cell lines, LINE-1 sequences were found to be significantly hypomethylated (Santourlidis et al., 2022).

Furthermore, histone modifications are another common epigenetic modification that is a biomarker in melanomagenesis (Besaratinia & Tommasi, 2014). Epigenetic modifications can happen in N-terminal tail domains, altering transcription and replication and causing malignant transformations (Fath et al., 2022). Out of the multiple histone modifications that have been reported in cutaneous melanomas, an increase in global levels of dimethylated histone H3 at lysine 9 has been found. Unfortunately, melanoma patients that have this histone modification have been associated with poorer survival outcomes (Santourlidis et al., 2022). Moreover, histone hypoacetylation has downregulated proapoptotic proteins, including the Bcl-2 family of proteins and the tumor suppressor gene PIP2, a negative PI3K/AKT signaling pathway regulator. Lastly, histone-modifying enzymes have demonstrated oncogenic potential. In particular, the histone methyltransferase SETDB1 is commonly amplified in melanoma, and it results in accelerated tumor development in melanoma models harboring the BRAFV600E mutation (Lee et al., 2014).

Oncogene Mutations and Affected Signaling Pathways

The three well-known and most common melanoma mutations occur in the oncogenes BRAF, NRAS, and NF-1, and they all play a critical role in the essential mitogen activated-protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling pathway. Dysregulation of the MAPK/ERK pathway, in this case constitutive overactivation, is one of the key events in melanogenesis.

The MAPK/ERK pathway begins when a cascade of protein kinase activity is activated by extracellular signals binding to cell membrane receptors, such as G-protein coupled receptors and receptor tyrosine kinases (RTKs) (Alqathama et al., 2020). Then, the rat sarcoma virus (RAS) family of proteins, which includes NRAS, KRAS, and HRAS, adopts its active state by the conversion of a GDP-bound state in RAS-GDP to RAS-GTP (Paluncic et al., 2016). Subsequently, the activated RAS GTPase binds in the membrane to activate its effector rapidly accelerated fibrosarcoma (RAF) proteins, which include the three isoforms ARAF, BRAF, and CRAF. Most commonly, following the activation of BRAF, a series of kinases are phosphorylated to activate their substrates, beginning with MEK1 and MEK2 (Kiuru & Busam, 2017). Then, MEK1 and MEK2 subsequently phosphorylates ERK1 and ERK2 MAPKs. MAPK proteins can translocate to the nucleus and regulate several transcription factors, consequently regulating several cellular mechanisms like cell proliferation and senescence, cellular death, differentiation, and cell survival (Paluncic et al., 2016).

While the MAPK/ERK signaling pathway is found to be dysregulated in more than 70% of all melanomas, this review will also delve briefly into the phosphoinositide 3-kinase / protein kinase B (PI3K/AKT) signaling pathway, one that is found to be dysregulated in more than 50% of all melanomas (Karachaliou et al., 2015).

PI3K is a lipid kinase that is involved in cell growth, cell proliferation, and metabolism. In the pathway, PI3K kinases are activated by binding insulin growth factor 1 (IGF-1) to IGF-1 receptors. The central role of PI3Ks is to catalyze the formation of phosphatidylinositol-3,4,5-triphosphate (PIP3), which then activates 3-phosphoinositide-dependent protein kinase 1 (PDK1). Subsequently, PDK1 phosphorylates and activates the serine-threonine-specific kinase AKT, inhibiting the substrate GSK3β, allowing free β-catenin proteins to accumulate and translocate to the nucleus. In the nucleus, free β-catenin up-regulates the expression of oncogenic genes, including c-MYC and cyclin D1, eliciting a strong anti-apoptotic effect and promoting cancer progression (Paluncic et al., 2016). Besides the oncogenic effects of the PI3K/AKT pathway, unfortunately, the pathway is an alternate path of melanoma resistance to BRAF inhibitor treatment (Karachaliou et al., 2015).

Lastly, phosphatase and tensin homolog (PTEN) is a PI3K/AKT pathway inhibitor; however, a loss of expression in PTEN has been observed in 30-50% of melanoma cell lines. PTEN functions by phosphorylating the conversion of PIP3 back to PIP2, resulting in reduced p-AKT levels, thus inhibiting the PI3K pathway (Paluncic et al., 2016). Interestingly, 70% of melanomas with mutated BRAF genes also exhibit inactivation or diminished expression of PTEN (Karachaliou et al., 2015). Furthermore, increased expression in the IGF-1 receptor caused by the loss-of-function of PTEN can cause BRAF inhibitor resistance in tumors (Karachaliou et al., 2015).

BRAF Mutations and Current Treatments

The BRAF mutation is the most common genetic mutation found in melanoma patients, and it is responsible for about 50% of cutaneous melanomas (Cancer.Net, 2023). Additionally, most BRAF mutations arise from intermittently sun-exposed skin (Karachaliou et al., 2015). As mentioned earlier, the BRAF gene encodes for RAF proteins, which are serine/threonine protein kinases including ARAF, BRAF, and CRAF isoforms, and they regulate several cellular mechanisms, including cell proliferation, differentiation, and survival (Alqathama et al., 2020).

The most frequent BRAF mutation, BRAFV600E, is a t1796A point mutation that results in the substitution of valine for glutamic acid in the second position of codon 600 of exon 15. Exon 15 is important for RAF enzyme activity and signaling through the MAPK/ERK pathway. BRAF mutations are characterized into two different categories: ones that cause RAS-independent activation of MEK1/2 and ERK and others that activate the CRAF gene (Karachaliou et al., 2015).

Emerging data indicates that these mutations lock BRAF into its active position, thus constitutively resulting in a ten-fold increase in oncogenic signaling through the pathway (Alqathama et al., 2020). In other words, the BRAFV600E mutation causes constitutive activation of the RAF kinase. Additionally, BRAF becomes insensitive to negative feedback mechanisms such as through the NF1 gene. BRAFV600E has been implicated in different mechanisms of melanoma progression, notably the evasion of senescence and apoptosis, unchecked replicative potential, angiogenesis, tissue invasion, and metastasis, as well as the evasion of immune response (Ascierto et al., 2012).

BRAF inhibitors were developed to target the mutant isoforms of RAF, particularly at the 600-codon position. While the development of these inhibitors has revolutionized the treatment of BRAF-mutant melanoma, unfortunately, the reality is that most patients develop disease progression after 6 or 7 months of inhibition, and only a small percentage of patients remain progression-free beyond a year (Karachaliou et al., 2015).

In most cases, melanoma disease progression continues because the MAPK/ERK pathway has become reactivated as a resistance mechanism to BRAF inhibitors. MAPK/ERK pathway reactivation can occur through mechanisms including alternative splicing forms of BRAFV600E, amplification of BRAFV600E, the acquisition of other activating mutations in NRAS or MEK, or the loss of function of NF1 through a mutation. Similarly, the body may overexpress MAP3K8, a kinase in the phosphorylation cascade, and this can drive resistance to treatment through ERK activation independent of RAF signaling. Lastly, the upregulation of RTKs that receive the initial signal to activate the MAPK/ERK pathway can lead to pathway reactivation and resistance to treatment (Karachaliou et al., 2015).

Another problem arises with the therapeutic approach of BRAF inhibitors: although they inhibit MEK/ERK signaling in BRAF mutant cells, they have a negative effect by activating MEK/ERK signaling in RAS mutant cells (Karachaliou et al., 2015). The presence of oncogenic RAS causes BRAF inhibitors to drive the formation of BRAF-CRAF hetero and homo-dimers, which activate CRAF and consequently stimulate MEK/ERK overactivation. It is important to note that most melanoma patients carry either a mutated BRAF *or* a mutated NRAS. However, the mutations are not mutually exclusive and can co-exist (Raaijmakers et al., 2016). An emerging treatment to combat resistance to BRAF inhibitors and activation of MEK/ERK signaling in RAS mutant cells has been to combine the targeting of BRAF and MEK1/2. The combination of dabrafenib, a BRAF inhibitor, with trametinib, a MEK1/2 inhibitor, has been approved by the FDA as a potential treatment for patients with mutant BRAF melanomas (Karachaliou et al., 2015).

NRAS Mutations and Current Treatments

Typically, melanoma patients with wild-type, unmutated BRAF have mutations in genes encoding upstream proteins, including mutations in NRAS (Paluncic et al., 2016). While NRAS was the first discovered oncogene in melanoma, it is the second most common oncogenic driver. Notably, NRAS mutations are found in 15-20% of melanomas, and unfortunately, NRAS-mutant patients experience more aggressive melanomas (with thicker lesions, elevated mitotic activity, and higher rates of lymph node metastasis) and consequently are associated with poorer survival outcomes (Muñoz-Couselo et al., 2017). Additionally, patients with mutant NRAS tumors tend to be older and have a history of chronic UV exposure (Muñoz-Couselo et al., 2017).

The RAS family of GTPases (of NRAS, KRAS, and HRAS, and mutations are present in 20%, 2%, and 1% of all melanomas, respectively (Muñoz-Couselo et al., 2017). NRAS is the most commonly mutated isoform of RAS in melanomas, and it has a point mutation leading to the substitution of glutamine for leucine

at position 61, with mutations at codons 12 and 13 being less frequent. NRAS mutations disrupt the GTPase activity of RAS, locking it in an active conformation. The constitutively active NRAS affects the MAPK/ERK pathway by inducing cell-cycle dysregulation, pro-survival pathways, and cellular proliferation, all leading to tumorigenesis (Muñoz-Couselo et al., 2017).

Although targeted therapies for BRAF-mutant melanomas are transforming the treatment of metastatic melanoma, there has been little progress in developing an ideal treatment for NRAS-mutant melanoma (Muñoz-Couselo et al., 2017). In particular, small-molecule antagonists of NRAS remain technically challenging to develop for numerous reasons: there is a lack of hydrophobic pockets deep enough to fit a small molecule, NRAS has a high affinity for GTP, and the high intracellular concentration of GTP is very high (Echevarría-Vargas & Villanueva, 2017). Moreover, another challenge to NRAS mutation treatment arises in melanomas with a lower tumor-infiltrating lymphocyte grade. Specifically, NRAS mutations have a more immunosuppressed microenvironment, potentially negatively affecting its response to immunotherapies (Karachaliou et al., 2015).

Despite these challenges, there are treatment options to combat NRAS-mutant melanomas. In one such treatment, an indirect approach to combating NRAS mutations includes blocking the horizontal and vertical signaling networks in the MAPK/ERK and PI3K/AKT pathways (Echevarría-Vargas & Villanueva, 2017). Another treatment option combines MEK inhibitors with agents inhibiting cell cycling and the PI3K/AKT pathway (Muñoz-Couselo et al., 2017). Novel treatments targeting KRAS, including RAS mimetics that prevent RAS/effector interaction and monobodies for the dimerization interface of KRAS, are showing promise as another strategy (Echevarría-Vargas & Villanueva, 2017). Lastly, NRAS-mutant patients had a better response to immunotherapy than patients with other genetic mutations, so immunotherapies, in particular immune checkpoint inhibitors, may be an effective future treatment option for NRAS-mutant melanoma patients (Muñoz-Couselo et al., 2017). Currently, patients with NRAS mutant tumors receive anti-programmed cell death protein 1 (PD1), anti-cytotoxic T-lymphocyte associated protein 4 (CTLA4), or anti-PD-L1 as immune checkpoint inhibitors, preventing cancer cells from signaling to T-cells and allowing T-cells to recognize and attack cancer cells (Echevarría-Vargas & Villanueva, 2017).

NF1 Mutations

Another co-existing and cooperative mutation in melanoma is the NF1 (neurofibromin 1) gene mutation (Cirenajwis et al., 2017). Mutations in NF1 are loss-of-function mutations that can occur from missense and nonsense mutations, frameshifts, deletions, translocations, and other forms. NF1 is a tumor suppressor gene that encodes a negative regulator of RAS, neurofibromin 1, a protein that controls cell growth by interacting with RAS in the MAPK/ERK pathway. Neurofibromin 1 negatively regulates RAS by converting the active RAS-GTP form into the inactive RAS-GDP form, which inhibits downstream RAS signaling (Kiuru & Busam, 2017). Mutations in NF1 result in a loss-of-function of neurofibromin 1, preventing essential negative regulation of the MAPK/ERK pathway.

Additionally, NF1 cooperates with mutated BRAF melanomas by preventing oncogene-induced senescence. So, for melanomas with NF1 mutations, studies have shown that this leads to decreased sensitivity and resistance to BRAF inhibitors (Cirenajwis et al., 2017).

Overview of Traditional Treatments

A summary of traditional treatments and current targeted therapies for melanoma will preclude this review's focus on novel and breakthrough immunotherapy options.

Traditional treatments used to combat melanoma include tumor excision surgery, and this option is primarily for non-metastasized tumors and is unviable for patients at advanced stages of the disease. Cytokine

interferon alpha 2b is an immunotherapy that possesses anti-tumor qualities. Additionally, interleukin-2 is a T-cell growth factor that inhibits tumor growth; however, since its introduction, it has been disregarded as a potential cure because of low response rates. Another traditional form of immunotherapy is the use of tumor-infiltrating lymphocytes, which involves the reinfusion of T-cells into the host body to target cancer cells. In regard to targeted therapies, immune checkpoint inhibitors (ICIs) have emerged as a class of standard treatment, and in particular, they have the potential to cure metastatic melanoma. As previously discussed, immune checkpoint inhibitors, including programmed cell death-1, cytotoxic T-lymphocyte antigen-4, and BRAF inhibitors, can be used to treat metastatic melanoma, and they are often used in conjunction with chemotherapy drugs (Jazirehi, 2021). Specifically, ICIs are monoclonal antibodies that target the specific receptors found on the surface of melanoma cells, disabling the deactivation of host cytotoxic T-lymphocytes that may be induced by cancer cells (Bafaloukos et al., 2023).

Proposal for Novel Melanoma Treatments

The heterogeneity of melanoma and the development of resistance to targeted treatments, through methods mentioned previously, provides a challenge to combating melanoma disease progression. So, while immunotherapy became a principal treatment for patients with non-chemosensitive melanoma, certain patients are still unresponsive to this approach. A clinical trial demonstrates that 50% and 54% of melanoma patients under ipilimumab and nivolumab ICIs will experience disease progression at 1 and 5 years of initial treatment, respectively (Bafaloukos et al., 2023).

Immunotherapy treatment strategies and related research have become increasingly common in recent years. Moreover, the limited success rates and development of resistance to traditional treatments and targeted therapies underscores the necessity of new, efficacious forms of treatment. For instance, using ICIs anti-CTLA-4 and anti-PD-1 is unresponsive in 50% of metastatic melanoma patients (Winge-Main et al., 2020). This review will explore two novel immunotherapy treatments, the use of T-cell receptor-engineered T-cells and the injection of a mRNA vaccine, and they may be considered unparalleled melanoma treatments, especially for metastatic patients.

TCR-Engineered T-Cell Immunotherapy

The novel TCR-Engineered T-cell immunotherapy (TCR-T) treatment method has emerged as a breakthrough for treating patients with advanced disease progression. This new immunotherapy-based treatment involves the ex vivo expansion and activation of antigen-specific T-cell receptor (TCR) engineered T-cells. Specifically, T-cells are engineered to have a high affinity for melanoma-specific antigens, and then they are reintroduced into patients to recognize and target the cancer cells expressing the antigens (Jazirehi, 2021).

Fortunately, TCR-engineered T-cells can recognize both extracellular and intracellular antigens (Liu et al., 2020). Several differentiation antigens have been strongly expressed in up to 95% of melanoma tumors, regardless of the stage (Winge-Main et al., 2020). Among these antigens, NY-ESO-1 has been found to be a safe and effective target, and more than 30% of clinical trials are targeting NY-ESO-1 (Liu et al., 2020). In addition, other commonly expressed antigens include tyrosinase, tyrosinase-related proteins (TRP-1 and TRP-2), melanocyte antigen (melan-A/MART-1), and glycoprotein 100 (gp100). Tyrosinase is expressed in about 80-90% of primary and metastatic melanomas. TRP-1 may have a role in melanocyte proliferation and death, and overexpression of TRP-2 contributes to melanoma resistance to chemotherapy and radiation therapy. It is important to note that melanoma-specific antigens may be lost or acquired in melanoma cells, highlighting the possibility for both the loss and gain of immune susceptibility and treatment response during the course of the disease (Winge-Main et al., 2020).

Originally, the remarkable capability of TCR-T was first demonstrated by a clinical trial that cured a metastatic melanoma patient with melanoma-associated antigen NY-ESO-1 specific CD4+ T-cells isolated directly from the patient. Moreover, the patient demonstrated clinical remission (Hunder et al., 2008).

Following that notable success, there are numerous ongoing in vivo TCR-T-based clinical trials and their results have validated TCR-T as a promising melanoma treatment option. This review will summarize the positive results of multiple clinical trials. Firstly, a trial targeting MART-1 resulted in a partial response in 6/24 patients, and another trial targeting the same antigen demonstrated tumor regression in 69% (9 out of 13) of patients (Winge-Main et al., 2020). A promising TCR-T clinical trial targets NY-ESO-1, and it demonstrated objective clinical responses in 55% (11 out of 20) of melanoma patients, and the estimated 3 and 5-year survival rates were 33% (Winge-Main et al., 2020). In another clinical study, Rosenberg's group targeted the antigen MAGE-A3, and cancer regression was seen in 4 out of 7 patients (Liu et al., 2020). In 2006, the Rosenberg research group reported that 12% (2 out of 17) of patients experienced tumor regression while treated with MART-1-specific TCR-T (Liu et al., 2020). Similarly, in another clinical trial, objective regression was noted in two patients treated with TCR-T specific for the antigen MART-1 (Morgan et al., 2006). Further studies indicated that 12% (4 out of 34) and 30% (6 out of 20) of patients had clinical responses while being treated with TCR-T using MART-1 specific antigen (Liu et al., 2020).

The nominal successes of these clinical trials demonstrate the significant promise that TCR-T has to become a treatment with high success rates and partial or complete melanoma remission in patients.

TCR-engineered T-cell immunotherapy has resulted in various responses, from ineffective results to complete, remarkable remission (Jazirehi, 2021). The basis of TCR-T is its specificity and high affinity for tumor-associated antigens. Needless to say, this method of immunotherapy will continue to play a role in cancer treatment, and the current positive clinical trial results hold an incredible amount of promise for treatment improvement.

Limitations and Challenges of TCR-Engineered T-Cell Therapy

Despite the promise TCR-T holds as a therapeutic strategy for melanoma treatment, it has its own limitations. The primary challenges facing TCR-T include that it can induce undesired negative side effects in the host, and patients can develop resistance to immunotherapy (Jazirehi, 2021).

Firstly, the most common adverse side effect of TCR-T is cytokine release syndrome (CRS). CRS occurs after the infusion of activated transgenic T-cells, resulting from the release of large quantities of cytokines that follow. Patients with CRS experience headaches, seizures, loss of memory, and loss of consciousness; however, severe toxicities may also occur, including life-threatening cardiac dysfunction, lung inflammation, severe neurological damage, and severe blood clots. Fortunately, IL-6 inhibitor tocilizumab is a common treatment for CRS, and it is used to dampen the effects of CRS (Jazirehi, 2021).

Furthermore, mutations in the major histocompatibility complex (MHC) can result in patients developing primary resistance to immunotherapy. A mutation of MHC in melanoma cells prevents TCR-engineered T-cells from identifying and binding to its specific antigens, preventing the evasion of tumor cells from immune system attack. Additionally, melanoma cells can downregulate beta-2 microglobulin, a regulator protein of the immune system, which allows them to proliferate rapidly and evade detection by T-cells (Jazirehi, 2021). Thus, patients deficient in beta-2 microglobulin will not respond to TCR immunotherapy.

Lastly, patients can develop acquired secondary resistance to immunotherapy through the overproduction of interferons. Generally, interferons function to increase the antitumor effects of the host immune system during immunotherapy. However, the overproduction of interferons can result in an upregulation of indolamine 2, 3 dioxygenase (IDO), which is an immune-based antitumor treatment. Interestingly, the upregulation of IDO allows cancer to thrive in a newly, antitumor-derived environment, leading to a rapid progression of tumors (Jazirehi, 2021). Nonetheless, it is important to understand that TCR immunotherapy remains an incredibly

hopeful method of treatment, and further research is needed to maximize its efficacy in treating metastatic melanoma patients.

Personalized mRNA Vaccine Immunotherapy

The experimental mRNA vaccine has emerged as a form of treatment, especially in combination with immunotherapy. In this treatment method, mRNA vaccines are administered ex vivo, when they are incubated with antigen-presenting cells isolated from the patient and then re-introduced into the host. Additionally, an alternative approach consists of direct administration of the mRNA vaccine to the patient, and both methods of infusion intend to provoke an immune response targeting the antigen-presenting melanoma cells. Although this review will not discuss recent technological advancement in detail, the development of lipid nanoparticles has been experimentally used as mRNA vectors that can transport mRNA into the cytoplasm (Bafaloukos et al., 2023).

The basis of an effective mRNA vaccine is vaccine stability, especially given mRNA's frail nature and the vast presence of extracellular degrading RNases. A robust mRNA vaccine can be created by incorporating 5' and 3' untranslated regions, preventing the vaccine's degradation. Other mRNA modifications may include capping the 5' area and attaching a polyA tail to the 3' area to stabilize the mRNA sequence (Bafaloukos et al., 2023).

Previous clinical trials have successfully produced and administered mRNA vaccines, and this report will first examine the results of animal model trials. In 2018, Wang's group worked with murine models, and they successfully performed an in vitro transfection of a mRNA vaccine, incorporating it into dendritic cells. The mRNA vaccine encoded for tyrosinase-related protein 2 (TRP-2) and silencing RNA (siRNA) targeting PD-L1 expression. TRP-2 mediates melanin synthesis in melanocytes, so it has been reported to confer melanoma cell resistance and survival when overexpressed. Following vaccination, the murine models generated increased CD8+ T-lymphocytes in the lymph nodes, tumor mass, and spleen. Additionally, tumor growth was delayed, and PD-L1 expression was effectively knocked down. Similarly, an in vivo study was performed with mRNA vaccines encoding gp100 and TRP-2, administered to murine B16F10 melanoma models. As a result, the vaccine-induced significant tumor shrinkage and prolonged survival of the treated mice (Bafaloukos et al., 2023).

In addition, clinical trials using mRNA vaccines have been undertaken with advanced melanoma patients. In 2006, a vaccine consisting of autologous monocyte-derived dendritic cells was intranodally or intradermally injected into 22 melanoma patients (Bafaloukos et al., 2023). A vaccine-induced immune reaction associated with T-lymphocyte expansion and interferon-γ production was observed in 9 out of 19 patients (Bafaloukos et al., 2023). Later, immune-specific CD4+ and CD8+ T-cell responses against neoantigens encoded by the mRNA vaccine were reported among 9 patients (Bafaloukos et al., 2023). In another instance, the TriMix mRNA vaccine, which consisted of mRNA encoding for CD40 and CD70 ligands, was tested in various trials. In one study, the TriMix vaccine, based on tyrosinase, gp100, MAGE-A3, and MAGE-C2 antigens, was combined with the use of ipilimumab in 30 advanced-stage melanoma patients (Bafaloukos et al., 2023). Significantly, there were reported 5-year overall survival rates and progression-free survival rates of 28% and 18%, respectively (Bafaloukos et al., 2023).

In February of 2023, the FDA recently granted breakthrough therapy designation to a new mRNA vaccine when administered with an adjuvant treatment, pembrolizumab (Conroy, 2023). The therapy designation comes after a substantially successful clinical trial, which showed that combining the mRNA vaccine and pembrolizumab reduced the likelihood of melanoma recurring or causing death by 44% compared to immunotherapy alone (Weber, 2023). The novel, personalized mRNA-4157/V940 vaccine consists of a single synthetic mRNA strand that codes for up to 34 neoantigens, specific abnormal proteins produced by cancer cells. The neoantigens are specific to a patient's tumor, and they are identified when researchers remove the tumors to analyze them for neoantigens. As a result, T-cells are produced specific to the neoantigen proteins encoded by

the mRNA, allowing the T-cells to specifically attack the patient's melanoma cells (Weber, 2023). Additionally, the mRNA vaccines mediate antigen presentation when they are incorporated by dendritic cells. Then, they induce the activation of cytotoxic CD8+ and CD4+ cells and release inflammatory mediators to trigger an immune response to fight the cancer cells (Bafaloukos et al., 2023). The vaccine has been used with the ICI pembrolizumab, and this has increased success rates.

On this note, mRNA vaccines may serve to overcome host resistance to ICIs. It has been found that melanoma tumors carrying a limited variety of neoantigens may surpass immune surveillance, so they are less responsive to ICIs. Fortunately, mRNA vaccines that encode immune-activation-associated molecules, including IL-12, interferon-alpha, GM-CSF, and TLR4, may be able to counter-balance the immune suppression induced by cancer cells. This would restore immune cell activity and inflammatory mediator production to the host's immune system (Bafaloukos et al., 2023).

It is important to investigate the possible improvements to mRNA vaccine-based treatments in the future. Firstly, it is necessary to identify highly immunogenic proteins that will allow effective and specific immune system stimulation without affecting cancer cells. Secondly, developing more stable mRNA vaccines that can escape early degradation is crucial to the less costly and widespread use of mRNA vaccines. Furthermore, it is important to understand possible combinations of the mRNA vaccines with ICIs, chemotherapy, or radiotherapy and the effectiveness of mRNA vaccines on disease relapse or progression prevention. Lastly, researchers should look into the clinical benefit of mRNA vaccines on metastatic tumors and as first- or second-line treatment options (Bafaloukos et al., 2023). Investigating these queries can inform and increase these vaccines' effective and safe use.

Conclusion and Future Prospects

Malignant melanoma is the deadliest form of skin cancer, and a significant amount of research has already been done to achieve breakthroughs in the current knowledge surrounding melanoma.

In terms of future directions, new detection and screening methods for melanoma should be considered. The earlier diagnosis of melanoma before cancer metastasis will substantially increase patients' survival outcomes and treatment efficacy.

Moreover, it is crucial that future research is directed toward improving the current treatments of ICIs and inhibitors that function in the dysregulated signaling pathways. Specifically, researchers need to investigate how to combat the resistance melanoma cells can develop to inhibitors. Another area of important investigation is the adverse reactions associated with TCR-T and a combination of TCR-engineered T-cells. Another major challenge that engineered T-cells must overcome is the highly immunosuppressive TME. A combination of ICIs, TCR-T, and mRNA vaccines may be the future of malignant melanoma treatment. Despite the tremendous improvements in melanoma treatments and survival rates, half the patients with stage IV melanoma experience disease progression (Winge-Main et al., 2020). This underscores the importance of new, efficacious immunotherapy treatments, and there is significant potential in both TCR-T and mRNA vaccines. They are already promising to be remarkably effective, and eventually, they may become the standard for melanoma treatment.

Acknowledgments

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