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The Modern Approach to Targeting Melanoma

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Abstract

Melanoma treatment depends largely on the clinical stage of the disease. The preferred treatment is surgical resection of the disease. The surgical margins depend on the depth of the disease. Sentinel lymph node biopsy is generally advised for all lesions greater than 1 mm depth. Complete lymphadenopathy of surrounding lymph nodes is recommended in the presence of nodal disease. There are some controversies surrounding the timing and benefit of complete lymphadenopathy in clinically occult disease. There is evidence to support the role of adjuvant therapies in the form of immunotherapy in regionally advanced disease, and there has been a significant improvement in medical therapies for advanced melanoma. BRAF inhibitors have become mainstay treatment for patients with a BRAF mutation. Immunotherapy is another cornerstone of therapy for advanced melanoma. There is ongoing research to define the optimal therapeutic regimen. Future guidelines will likely incorporate this recent research. Chemotherapy has been relegated to second-line therapy in melanoma.

Keywords: melanoma, staging, therapeutic advances, immunotherapy, chemotherapy

1. Introduction

Melanoma is the deadliest of all the skin cancers. The incidence has been increasing in recent decades. There has been a significant development of therapies for melanoma. For the purpose of chapter, the staging of melanoma will be reviewed. There will be a brief overview of the current recommendations and ongoing research into the different therapeutic approaches.

2. Determining the appropriate therapy

An important determinant of the appropriate treatment strategy is the clinical stage of the primary melanoma. Melanoma is staged by the TNM system. T refers to the primary tumour, N refers to the nodal status and M refers to the metastatic status. The AJCC released updated guidelines for the staging of melanoma in 2016. T1a tumours are less than 0.8 mm deep without ulceration, and T1b tumours are either less than 0.8 mm deep with ulceration or 0.8 mm to 1 mm deep with or without ulceration. T2a and T2b tumours are 1.0 mm to 2.0 mm without and with ulceration, respectively. T3a and T3b tumours are 2.0 mm to 4.0 mm without and with ulceration, respectively. Finally, T4a and T4b tumours are greater than 4.0 mm without and with ulceration, respectively. N1 refers to one-tumour involved or any number of in-transit satellite and/or microsatellite metastases with no tumour-involved lymph node. N1a and 1b refer to one clinically occult (detected by SLNB) and one clinically detected. N2 refers to two or three tumour-involved nodes or any number of in-transit, satellite, and/or microsatellite metastases with one tumour-involved node. N2a and N2b refer to two or three clinically occult and at least one clinically and one clinically occult/clinically detected, respectively. N3 refers to four or more tumour-involved nodes or any number of in-transit, satellite, and/or microsatellite metastases with two or more tumour-involved nodes or any number of matted nodes without or with in-transit, satellite and/or microsatellite metastases. N3a, N3b and N3c refer to four or more clinically occult, four or more, at least one of which was clinically detected or the presence of any number of matted nodes or two or more clinically occult or clinically detected and/or the presence of any number of matted nodes, respectively. Satellite metastases are clinically apparent cutaneous and/or subcutaneous metastases within 2 cm of the tumour. Microsatellites are microscopic cutaneous and/or subcutaneous metastases next to or below the melanoma on histological examination. In-transit metastases are clinically apparent cutaneous and/or subcutaneous metastases found greater than 2 cm from the primary melanoma. M0 refers to no distant disease, and M1 refers to distant disease. M1a refers to distant metastases in the skin, soft tissue including muscle, M1b refers to distant metastasis to lung, M1c refers to distant metastasis to non-CNS visceral sites and M1d refers to distant metastasis to the CNS. M can be further risk stratified based on the LDH level [1].

Stage 1 and 2 are defined by tumour size. Stage 3 is defined by positive nodal disease. Stage 4 is defined by the presence of metastases. Based on the TNM staging, stage 0 refers to T1sNoMo, stage 1a refers to T1a/N0/M0, stage 1b refers to T1b or T2a/N0/M0, stage 2A refers to T2b or T3a/N0/M0, stage 2B refers to T3B or T4a/N0/M0, stage 2C refers to T4b/N0/M0, stage 3 refers to any T/N1 or higher/M0 and stage 4 refers to any T/any N/M1 [1].

2.1. Stages 0, 1 and 2

The ESMO guidelines recommend surgical therapy as the primary management strategy in localised melanoma. The guideline recommends against performing routine lymphadenectomy or irradiation to the surrounding lymph nodes. The guideline recommends radiotherapy for local disease control where there are positive margins in lentigo maligna melanoma, in cases of metastases resection where there are positive histological margins or after the removal of bulky disease [2].

2.1.1. Surgical

Surgical excision forms the cornerstone of treatment in primary cutaneous melanoma. Following the initial histological diagnosis and microstaging of the tumour, a wider and often deeper excision of the melanoma is performed. The surgical management of melanoma depends primarily on the Breslow thickness, the presence or absence of ulceration, and positivity of sentinel lymph node biopsy [3]. The ultimate aim of surgical therapy irrespective of depth is to obtain clear margins. The extent of the surgical margins is determined by three factors: (1) Wide margins result in a reduced risk of local recurrence. (2) There is no benefit to excising margins beyond 1 cm in thin melanoma. (3) There is no demonstrated benefit to excising margins beyond 2 cm in any thickness melanoma. The guidelines suggest a margin between 1 cm and 2 cm for primary cutaneous melanoma. Furthermore, the guidelines state that an excision should be performed to the level of muscle fascia or depending on tumour location at least to the level of the deep adipose tissue. However, in the case of stage 0 melanoma, an excision margin between 0.5 and 1.0 cm margin is acceptable. In the case of stage 0 lentigo maligna, margins may need to be extended to >0.5 cm, due to characteristically widespread subclinical extension. Permanent section total peripheral margin control and Moh's micrographic surgery have been used to achieve histological control of the margins. However, there is a paucity of evidence to support their use [4].

There are several trials performed to determine the optimal extent of the margins when excising melanoma. In thin melanomas, an international, randomised prospective study examined 1 cm margins in the context of primary cutaneous melanomas less than 2 mm depth. A total of 612 patients were included in the trial, with 305 randomised to 1 cm margins and 307 randomised to wide margins less than 3 cm. The disease-free and overall survival was similar in the two groups [5]. In the case of intermediate-thickness tumours (1 to 4 mm depth), a large, multicentre randomised trial demonstrated that margins of 2 cm were acceptable with respect to 5-year survival. Reducing the margins from 4 to 2 cm led to a significant reduction in skin grafting and total length of hospital stay [6]. In another multicentre trial, they investigated the optimal excision margins in high-risk melanoma. High-risk melanoma was defined as localised melanoma 2 mm or greater in thickness on the trunk or limbs. Elective lymph-node dissection, sentinel biopsy and adjuvant therapies were not permitted. Patients were either randomised to 1 cm or 3 cm margins. The trial demonstrated that a 1 cm margin in high-risk melanoma is associated with a significant increase in regional recurrence vs. a 3 cm margin, but both patient populations had a similar overall survival rate [3]. A Cochrane review examined the different excision margins in melanoma. A narrow margin was defined as 1–2 cm, and a wide margin was defined as 3–5 cm. The systematic review included data from randomised trials for 1633 participants in the narrow excision margin group and 1664 in the wide excision margin group. There was no statistically significant difference in terms of overall survival and recurrence free survival between wide and narrow margins. However, there was a trend toward improved overall survival and recurrence free survival. The review concluded that there was inadequate evidence to determine the optimal excision margins for primary cutaneous melanoma [7].

Sentinel lymph node biopsy should be considered in patients with a primary cutaneous melanoma of 1 mm or greater depth. Sentinel lymph node biopsy is generally not advised in

patients with stage 0 or stage 1a melanoma. The use of sentinel lymph node biopsy is only recommended in stage 1B in the presence of adverse prognostic indicators [4]. The likelihood of a positive sentinel lymph node biopsy increases with the Breslow's depth, with 2% in 1 mm, 7% in 1–1.99 mm, 13% in 1–1.99 mm, and 31% in 3 mm. In 710 cases of sentinel lymph node biopsy, 638 (88.5%) were alive without evidence of disease [8].

Lymph node dissection plays an important role in the surgical management of melanoma. The MSLT-1 was a multicentre phase 3 trial, which randomised two groups of patients with localised melanoma more than 1 mm deep to receive either wide excision with lymphatic mapping and sentinel lymph node biopsy with immediate complete lymphadenectomy for sentinel node metastases or wide excision plus postoperative observation with a deferral of the complete lymphadenectomy until clinically evident disease became apparent. The trial comprised of 1270 patients with intermediate-thickness melanoma, 290 with thick melanoma, and 232 with thin melanoma. There was no difference between the immediate and delayed complete lymphadenectomy group in the absence of nodal disease in either the intermediate or thick melanomas group with respect to 10-year melanoma-specific survival rates. The survival rate was much improved in the presence of nodal disease in the biopsy vs. observation group in the intermediate thickness group. However, a similar benefit was not observed in the thick melanoma group. The patients with a positive sentinel lymph node biopsy had a worse prognosis vs. the patients with negative sentinel lymph node biopsy. Thus, there is a clear benefit to immediately complete lymphadenectomy following the identification of clinically occult disease in intermediate-thickness melanoma with respect to nodal recurrence, distant metastases and melanoma-specific survival. The timing of the complete lymphadenectomy does not appear to play an important role in thick melanomas. There is no benefit to immediate complete lymphadenectomy in the absence of clinically occult disease. Finally, the trial demonstrates that sentinel lymph node biopsy serves as an important prognostic tool [9].

Another important trial which assessed the role of complete lymph node dissection was the phase 3, multicentre, DeCOG-SLT trial. They examined whether complete lymph node dissection results in a better overall survival vs. conservative management in patients with positive sentinel lymph node biopsies. The patients had cutaneous melanoma of at least 1 mm depth and positive sentinel lymph node biopsies. The trial randomised 483 patients to either complete lymph node dissection or observation. There was no significant difference in terms of distant-metastasis free survival in the treatment or observation arm (74.9% vs. 77.0%). Furthermore, there was no significant difference in terms of 3 year overall survival between the treatment and observation arm (81.2% vs. 81.7%). There was only a small improvement in disease control in the treatment vs. the observation group (8% vs. 15%). It is important to note that the majority of patients in that study had a low tumour burden [10].

2.1.2. Non-surgical

Surgical management is the treatment of choice for primary cutaneous melanoma. Hence, the non-surgical options should only be advised in specific cases, where surgery is not possible. The options include topical imiquimod, radiation therapy, cryosurgery and observation [4].

2.2. Stage 3

The ESMO guidelines suggest surgical excision and removal of the surrounding lymph nodes. They note that it is not sufficient to merely remove the disease-containing nodes. These guidelines define high-risk situations as the presence of multiple bulky lymph node metastases. The surgical management of stage III melanoma follows the same principles as above. The guidelines suggest the consideration of localised radiation therapy to the surrounding area in the case of high-risk disease. In the presence of inoperable, regionally advanced disease, the guidelines suggest therapies such as isolated limb perfusion, radiation therapy, electrochemotherapy or intralesional therapy [2].

In the presence of high-risk disease, systemic therapy should be considered. A recent phase 3 trial investigated the role of adjuvant immunotherapy in high risk regionally advanced to prevent recurrence. The trial entitled EORTC 18071 enrolled participants who underwent a surgical excision of cutaneous melanoma with clear margins. A total of 951 patients were randomly assigned to the treatment with either placebo or ipilimumab. The median recurrence survival was significantly improved in the ipilimumab treatment arm (26.1 months vs. 17.1 months). There were a large number of patients who discontinued ipilimumab due to adverse events (245/471). These adverse events were most commonly GI (75/472), hepatic (50/472), and endocrine (40/471). There were five treatment-related deaths in the ipilimumab treatment arm (three due to colitis, one due to myocarditis and one due to Guillain-Barré syndrome) [11]. The overall 5-year survival in the ipilimumab treatment arm at 5 years was 65.4% vs. 54.4% in the placebo arm [12].

However, the role of ipilimumab as the optimal adjuvant therapy has recently been challenged with the publication of a phase 3 trial, comparing adjuvant nivolumab vs. ipilimumab in resected stage 3 or 4 melanoma. A total of 906 patients were randomly assigned to either receive treatment with ipilimumab or nivolumab. The patients were followed up for at least 18 months. When the 12-month recurrence-free survival was compared in both groups, it was significantly higher in the nivolumab group (70.5% vs. 60.8% respectively). Nivolumab appeared to have a better overall side-effect profile, with 14.4% reporting grade 3 or 4 adverse treatment effects vs. 45.9% in the ipilimumab-treatment arm. Furthermore, while there were two deaths reported in the ipilimumab arm, there were no deaths recorded in the nivolumab arm [13].

A recent phase 3 trial published in 2017 suggests that the BRAF inhibitor dabrafenib plus the MEK inhibitor trametinib prove the optimal treatment option in stage III melanoma patients with the BRAF V600 mutations. A more detailed discussion regarding the mechanism of these drugs will be discussed in the stage IV section. They randomised patients with either a BRAF V600E or V600 K mutation to receive either a combination of dabrafenib and trametinib or placebo. They recruited 870 patients with adequately resected stage 3 melanoma. The overall 3-year survival was improved in the treatment arm vs. placebo (86% vs. 77%). The 3-year relapse free survival was 58% vs. 39% in the treatment vs. placebo arm, respectively. This trial suggests a role for combination BRAF/MEK therapy in stage 3 melanoma [14].

In conclusion, surgery with removal of surrounding lymph nodes remains the mainstay therapy for stage III disease. Several recent publications suggest a benefit to systemic therapy with checkpoint inhibitors or BRAF inhibitors if applicable in high-risk disease. Future guidelines will likely incorporate this recent research into their treatment strategies for regionally advanced disease.

2.3. Stage 4

The most significant breakthrough in melanoma in recent years has included the therapies designed for metastatic malignant melanoma. The main determinant of treatment strategy is the presence of resectable or unresectable disease. In the presence of resectable disease, the disease may be managed as the above. In the case of unresectable disease, the ESMO guidelines note that the optimal 1st-line therapy in melanoma remains under considerable debate. They suggest either anti-PD1 therapies or BRAFi/MEKi for BRAF-mutated melanomas. Chemotherapy has been relegated to 2nd-line therapy in the guidelines. However, in the case of aggressive metastatic disease, the guidelines note some benefit to polypharmacy, containing paclitaxel and carboplatin/cisplatin, vindesine, and dacarbazine [2]. Furthermore, radiation therapy is recommended in the presence of symptomatic brain metastases or painful bony metastases. However, the guidelines will likely evolve dramatically following the development of further therapeutic strategies.

2.3.1. MAPK pathway inhibitors

Dysregulation of the RAS/RAF/MEK/ERK MAPK pathway plays a pivotal role in the development of MM. In healthy cells, this pathway regulates several physiological cellular processes. The MAPK pathway is activated by growth factors which bind to the extracellular kinase receptor. This receptor-ligand complex leads to autophosphorylation of intracellular domains, which in turn results in phosphorylation and activation of the membrane-bound guanosine triphosphatase RAS. There is dimerisation of the serine/threonine kinases RAF. RAF is encoded by three different isoforms: ARAF, BRAF, and CRAF. The BRAF isoform encodes for the most powerful activator of the MAPK pathway. Activation of RAF causes a phosphorylation cascade, with the eventual activation of ERK. ERK is then free to translocate to the nucleus, where it activates several transcription factors that induce the expression of genes implicated in normal cell turnover and survival [15, 16].

In 2002, there was an exciting discovery that activating mutations in MAPK pathways play an essential role in most MM. A dysregulated MAPK pathway is present in ~40–50% of MM cases. The most common mutation resulting in dysregulation of the MAPK pathway is present on exon 15 and results in the switching of glutamate for valine at codon 600 (V600E). This mutation is located within the activating segment of the kinase domain. The mutant form of BRAF is more potent than the wild type variant. The mutant form results in constitutive activation of the MAPK pathway and increased ERK. The cellular endpoint is increased turnover and survival. The presence of BRAF mutations seems to be dictated by age; with 80% of patients less than 30 years old harbouring a mutation, while only 20% of patients over

80 years old harbouring the mutation. Furthermore, older patients are less likely to have the V600E mutation. Finally, patients with the mutant BRAF had historically worse outcomes than the wild-type BRAF [16].

As a result of this new understanding of the underpinning genetic events that give rise to MM, there have been several drugs developed known as targeted therapy of the MAPK pathway. Initially, the broad spectrum tyrosine kinase, sorafenib, was trialled in melanoma patients [17]. The clinical trial results proved disappointing. There are two targeted therapies subsequently developed and currently licenced, which inhibit mutated BRAF: vemurafenib (formerly known as PLX4032) and dabrafenib. Vemurafenib was the first selective tyrosine kinase inhibitor licenced by the FDA in 2011. Dabrafenib is a potent and selective inhibitor of BRAF V600E kinase. Inhibition of the pathway may also be achieved by MEK inhibitors. Trametinib is a potent and selective inhibitor of MEK 1 and 2. These three MAPK targeted therapies are licenced by the US Food and Drug Authority for single-agent therapy against non-resectable or metastatic cutaneous MM. Combining MAPK pathway inhibitors is an important therapeutic strategy to minimise the development of drug resistance. There is an additional MEK inhibitor, known as cobimetinib licenced for combination therapy with vemurafenib. Similarly, dabrafenib and trametinib are licenced for combination therapy [18, 19]. BVD-523 (ulixertinib), an ERK1/2 inhibitor with high potency and selectivity, is currently under investigation. Preclinical investigations *in vivo* and *in vitro* appear promising, and clinical trials are underway. This may prove an important combination therapy or refractory in future clinical practice [20].

An important therapeutic limitation is the development of resistance to the MAPK pathway inhibitors. The current literature suggests that progression-free survival for patients receiving BRAF/MEK inhibitor combinations ranges from 9 to 11 months [21–23]. The exact mechanism of resistance has not been fully elucidated, and there is ongoing research into the development of resistance. Several different mechanisms of resistance have been posed in the literature.

3. Immunotherapy

As seen in the previous section, melanoma is a highly mutated cancer. Similarly, it is extremely an immunogenic cancer. Attenuating the immune system has proved an important therapeutic strategy. Immunotherapy targets 4 broad areas: (1) Checkpoint inhibitors. These agents negatively regulate inhibitors of pre-existing anti-tumour immune response (effectively augmenting the response of the immune system to the tumour cells, e.g., Anti-CTLA-4 inhibitors (ipilimumab), anti-PD-1/PD-L1 antibodies (nivolumab/pembrolizumab/atezolizumab) and IDO1 Inhibitors (Epacadostat); (2) Increasing the anti-tumour T cell response by administration of autologous ex-vivo augmented tumour infiltrating lymphocytes; (3) Administering oncolytic viruses into the metastatic cells to break-up the cells and increase the immune response and (4) Targeting dendritic cells to start and/or increase tumour antigen-specific immune responses [24].

In order to appreciate the mechanism of the immunotherapies, it is necessary to briefly examine the normal physiology of the immune system. T cells are a subgroup of lymphocytes mainly produced in the thymus, which express antigen-recognising T-cell receptor (TCR). Every T-cell has a unique TCR, which recognises a specific antigen. Antigen is presented to the T-cell by the MHC complexes. This causes clonal expansion of T-cells. All progeny of a T-cell express the same TCR, and this expanded pool confers antigen-specific immunity. There are 4 main reasons why the T-cells are such a desirable target for immunotherapy: (1) The response to T-cell is specific, and differentiates between healthy and neoplastic cells; (2) T-cell responses are amplified, resulting in a 1000-fold increase in the response after activation; (3) T-cells travel to the specific area containing the antigen, enabling them to fight distant metastases; and (4) T-cells can remain quiescent, conferring immunity for many years after the initial exposure to antigen [25]. T cells may either be classed as effector T cells or T regulatory cells. Effector T-cells enable immunity and destroy cells with the particular antigen. T regulatory cells are essential for maintaining immunological unresponsiveness to self-antigen and preventing excessive immune responses harmful to the host. There are several different theories about how regulatory T-cells can attenuate the immune response: 1. Secretion of immunosuppressive cytokines by regulatory T cells, cell-contact dependent inhibition, and modification/destruction of APC cells [26–28].

The key molecules required for the activation of both T cell types are similar. T cell activation is mediated by activation of the T-cell receptor and a co-stimulatory molecule, the CD28 receptor on the T cells by MHC peptides, and APC. Both are necessary for generation of an adequate immune response. Antigen is presented to the TCR, and a T-cell receptor complex including CD3, CD2, CD4/CD8/LFA1/CD45R is formed. This activation of TCR generates signal 1. CD28 on T cells is activated by B7.1 and B7.2 on antigen-presenting cells, generating signal 2. B7.1 and B7.2 are generally only expressed on specialised antigen presenting cells, e.g., dendritic and Langerhans. Cytotoxic T-cell associated antigen-4 (CTLA4) and glucocorticoid-induced TNF receptor (GITR) are expressed on T cells. CTLA4 combines with B7.1 and B7.2 and blocks activation of the CD28 molecules. CTLA4 has a greater affinity for B7.1 and B7.2 than CD28. CTLA4 sends a negative signal, downregulating expression of B7.1 and B7.2 [26–28].

3.1. Checkpoint inhibitors

Checkpoint inhibitors are based on the fact that T lymphocytes are essential for the antitumour immunity. Furthermore, an antigen-specific TCR must be activated in the presence of co-stimulatory activation in order to activate the T-lymphocytes [29]. Several inhibitory receptors and ligands present on T cells and tumour cells have been identified as potential targets for cancer immunotherapy. They are essential mediators of immune suppression in the tumour microenvironment [30]. They are different from monoclonal antibodies, which bind and destroy the tumour cells. Checkpoint inhibitors are immunomodulatory antibodies which either stimulate or inhibit the function of cell surface signalling molecules on the patient's own immune cells. This can lead to either upregulation or downregulation of the patient's immune cells [31]. Different negative co-stimulatory molecules exist. Targeting negative co-stimulatory molecules, such as CTLA4 and PD-1, is the basis of checkpoint inhibitors. The advantage of checkpoint inhibitors is that they function irrespective of the patients' BRAF status [32].

3.1.1. CTLA-4 inhibitors

CTLA-4 blocking antibodies prevent CTLA-4 from binding to its ligands B7-1 and B7-2 on APCs. This “unleashes the breaks” on the immune system. Experimental evidence suggests that there is an increase in the absolute number of effector and regulatory T cells in the lymph nodes. However, there is an increase in the effector T cell to regulatory T cell ratio in the tumour microenvironment. Destruction of the regulatory T cells increases the ratio and directly correlated with rejection of the tumour. Another important mechanistic aspect of CTLA4 inhibitors is their effect on FcyR. FcyRs are responsible for the selective depletion of the regulatory T cells. FcyRs are key regulators of the immune response. FcyR is broadly expressed on cells of haemopoietic lineage, including B cells, macrophages, mast cells, NK cell and neutrophils. They can be both activating and inhibitory. Depletion of the T cells results from antibody-dependent cellular cytotoxicity, dependent on tumour-infiltrating CD11b-positive macrophages expressing activating FcyRIV. CTLA4 stimulates activating FcyRIV, inducing the antibody-dependent cellular cytotoxicity. The depletion of regulatory T cells occurs preferentially in the tumour cells for several reasons. Macrophages are a lot more plentiful in the tumour microenvironment (>50 times). Furthermore, there is a greater consumption of the T regulatory cells due to the higher expression of CTLA4 on T regulatory cells vs. T effector cells. This ultimately leads to a higher effector T cell to regulatory T cell ratio in the tumour [31, 33].

In 2010, the CTLA4 inhibitor, ipilimumab, proved to improve overall survival in the groundbreaking phase III clinical trials in patients with advanced melanoma. A total of 676 patients were included in the study with either stage III or IV melanoma. They randomised patients to receive ipilimumab, ipilimumab plus gp100, or gp100 alone (control group). The overall survival of patients who received ipilimumab vs. the control group was 10.0 months vs. 6.4 months. There was no survival difference in overall survival in patients receiving ipilimumab vs. ipilimumab plus gp100. Ipilimumab alone resulted in the best overall response (10.9%) and disease control rate (28.5%). A total of 60% (n = 9/15) of patients receiving ipilimumab alone had a long-term response lasting more than 2 years. It is important to note that 10–15% of participants receiving ipilimumab suffered from grade 3 or 4 immune-related adverse events, most commonly relating to the skin and gastrointestinal system [34]. In clinical trials, greater than 80% of participants experienced adverse events related to therapy with ipilimumab [35]. The most frequent severe immune-mediated adverse effects are enterocolitis, hepatitis, dermatitis (including TEN), neuropathy, and endocrinopathy. These adverse effects generally occur during treatment; however, they may occur in the weeks to months after ipilimumab discontinuation [35]. A pooled analysis was undertaken on the long-term survival data from phase II and III clinical trials, in patients with unresectable melanoma. The data included 1861 patients from 10 prospective and 2 retrospective trials. The median overall survival for patients receiving ipilimumab was 11.4 months. There were 254 patients with 3-year survival follow-up. The 3-year survival rate was 22% [36].

When ipilimumab was combined with dacarbazine, the median duration of the best overall response was 19.3 months vs. 8.1 months in dacarbazine alone at long-term follow-up [30].

EORTC 18071 was a multicentre phase 3 clinical trial with 951 patients comparing adjuvant ipilimumab (dose of 10 mg/kg) with placebo in resected, high-risk stage III melanoma. The overall survival rate at 5 years was significantly higher in the ipilimumab group vs. placebo (65.4% vs. 54.4%). The rate of distant metastasis-free survival at 5 years was increased in the ipilimumab vs. placebo (48.3% vs. 38.9%). 98.5% (n = 465/471) of patients receiving ipilimumab experienced an adverse event of any grade, and 26.2% experienced a grade 3 or 4 adverse event, with 41.6% of patients experiencing grade 3 or 4 immune-related adverse events in the ipilimumab group. The most common immune-related adverse events were GI, hepatic, and endocrine. Five patients (1.1%) died due to adverse events related to ipilimumab [12].

3.1.2. PD-1 inhibitors

The programmed death-1 (PD-1) is a regulatory molecule which is expressed on T cells and operates during the effector phase of T-cell activation. In contrast, CTLA-4 is operational during early activation of T cells in lymphatic tissues. PD-1 interacts predominantly with its two ligands, B7-H1 and B7-DC (PD-L1 and PD-L2) in peripheral tissues, and causes apoptosis and downregulation of T-cell effector function. The function of PD-1/PD-L1 interaction is to minimise the risk of surrounding tissue damage by T-cells. PD-1/PDL-1 therapies are considered “tumour site immune modulation therapy”. PD-L1 appears to be upregulated in the tumour microenvironment [30]. In order to extravasate peripheral tissues and organs, the T-cells must have encountered their antigen already in the lymphoid organs. T-cells primed with its antigen develop an immunologic memory and acquire a particular set of adhesion molecules that allow extravasation to the peripheral tissues, including the tumour. Once this T-cell encounters the antigen in the peripheral environment, PD-1 interacts with its ligands and thereby decreases the extent of the immune response. Therefore, PD-1 inhibitors target T-cells already engaged in the ongoing effector T-cell response and hence have a more restricted spectrum of T-cell activation compared with CTLA-3 blocking. This is probably the reason why there is a decreased rate of immune adverse events with anti-PD-1 or anti-PDL-1 antibodies [30].

In 2015, a phase 3 multicentre clinical trial investigated 518 patients with BRAF-negative advanced stage III or IV melanoma randomised to either nivolumab (dose 3 mg/kg) or dacarbazine. At 1 year, the overall survival rate was 72.9% in the nivolumab group vs. 42.1% in the dacarbazine group. The median progression-free survival was 5.1 months in the nivolumab group vs. 2.2 months in the dacarbazine group. The objective response rate was 40% in the nivolumab group vs. 13.9% in the dacarbazine group. Therapy-related adverse effects occurred in 11.7% of the nivolumab group vs. 17.6% in the dacarbazine group. The most common effects included fatigue, pruritus, and nausea [37]. In another clinical trial, an analysis was performed on the safety data relating to nivolumab in both melanoma and other solid tumour groups (n = 306). Some patients were followed-up for safety monitoring over 2 years. The majority of adverse events occurred in the first 6 months of treatment. There were no cumulative toxicities with extended treatment periods [38].

In CHECKMATE 037, a phase-3 multicentre clinical trial, 631 patients with advanced melanoma who progressed after CTLA-4 inhibitor were randomised to receive nivolumab or

chemotherapy as second-line or later-line therapy. Confirmed objective responses were recorded in 31.7% (n = 38/120) in the nivolumab group vs. 10.6% of the investigators' choice chemotherapy group (n = 5/47). Grade 3 and 4 drug-related serious events occurred in 5% (n = 12) of nivolumab-treated patients vs. 9% (n = 9). The grade 3 and 4 toxicities of nivolumab included deranged alanine aminotransferase, anaemia, and fatigue. There were no treatment-related deaths in this group [39].

Pembrolizumab is another PD-1 inhibitor used in clinical practice. KEYNOTE-006, a multicentre phase 3 clinical trial, including 834 participants compared pembrolizumab vs. ipilimumab. They excluded participants who received prior checkpoint inhibitor therapy. The 6-month progression-free survival rates were 47.3% for pembrolizumab every 2 weeks, 46.4% for pembrolizumab every 3 weeks, and 26.5% for ipilimumab. Twelve-month survival rates were 74.1, 68.4 and 58.2%, respectively. The response rate was higher with pembrolizumab, every 2-weeks (33.7%) and 3-weeks (32.9%) vs. ipilimumab (11.9%); 89.4, 96.7 and 87.9% had a sustained response, with a median follow-up of 7.9 months. There was a reduced rate of treatment-related adverse effects of grade 3–5 severity in the pembrolizumab group (13.3 and 10.1%) vs. the ipilimumab group (19.9%). Thus, the efficacy in both pembrolizumab groups was significantly higher than ipilimumab, with reduced treatment-related adverse events [40]. Follow-up of KEYNOTE-006 in 2017 showed overall superiority and progression-free survival of pembrolizumab vs. ipilimumab. The median follow-up was 22.9 months. The 24-month overall survival rate was 55% in the 2-week group, 55% in the 3-week group, and 43% in the ipilimumab group. Most immune-related events occurred within the first 6 months of therapy. Colitis was more common in the ipilimumab group, whereas hepatitis and endocrinopathies were more common in the pembrolizumab group [41]. About 19% (n = 38) treated with pembrolizumab for at least a year developed grade 3–4 treatment-related adverse events. No patients died because of the therapy-related toxicity.

There is ongoing work investigating the role of atezolizumab in advanced melanoma. In a phase 1b trial, atezolizumab was combined with vemurafenib in BRAFV600 metastatic melanoma in 17 patients. It produced an overall response rate of 76%, with three complete responses and 10 partial responses. About 41% experienced grade 3 treatment-related adverse effect and no participants experienced a grade 4 adverse effect or death. Further work is necessary to elucidate the role of atezolizumab in melanoma [42].

A recent analysis was performed on the safety data from 48 trials (n = 6938), including 26 CTLA4, 17 PD-1, 2 PD-L1 trials and 3 CTLA4 and PD1. There were more grade 3/4 immune-related adverse events with CTLA4 inhibitors vs. PD-1 (31% vs. 10%). Colitis, hypophysitis and rash were more common with CTLA4 inhibitors, whereas pneumonitis, hypothyroidism, arthralgia, and vitiligo were more common with PD1 inhibitors. Melanoma patients specifically have a higher incidence of gastrointestinal and skin immune-related adverse effects and a reduced incidence of pneumonitis. The discontinuation rate to immune-related adverse effects was between 3 and 12% in anti-PD-1 trials and 3 and 25% in anti-CTLA4 trials. The most frequent cause of discontinuation was diarrhoea/colitis. Death was an extremely uncommon event for anti-PD1 agents (pembrolizumab 0.1%, nivolumab 0.3%) and mostly occurred

due to pneumonitis. Death occurred in 29 patients receiving CTLA-4 inhibitors and was more often due to gastrointestinal events such as diarrhoea, colitis, and colonic perforation [43].

3.1.3. IDO1-inhibitors

Indoleamine 2,3-dioxygenase (IDO-1) is a significant immunoregulatory enzyme that facilitates immunosuppression, tolerance, and tumour evasion by tryptophan catabolism [44]. It is a molecule which causes oxidative cleavage of tryptophan, an amino acid which is essential for cell proliferation and survival. IDO1 induction triggers dendritic cell apoptosis and inhibits T-cell response. In multiple cancer types, the IDO1 pathway is activated. In vitro, inhibition of IDO1 causes an increase in T and natural killer cells, increase in IFN-production, and reduced switch to regulatory T-cells [45].

Epacadostat is the first IDO-1 inhibitor in its class. Experimental evidence suggests that T-cells stimulated with dendritic cells treated with epacadostat produce a greater number of inflammatory mediators. Furthermore, there appears to be a decrease in the number of regulatory T cells [44]. Echo-202/KEYNOTE-037 was a phase 1 clinical trial, which enrolled 62 patients with advanced melanoma. Patients treated previously with checkpoint inhibitors were excluded. The patients received epacadostat plus pembrolizumab. Grade 3 or higher treatment-related adverse effects occurred in 18%, with the most common being rash, followed by increased lipase. No treatment-related deaths occurred. There were four complete responders, seven partial responders and three stable disease noted [46]. There were phase I/II trials with 40 advanced melanoma patients investigating the tolerability and efficacy of epacadostat plus ipilimumab. Grade 3 or higher immune-related treatment effects occurred in 23% of participants, rash, pruritus, diarrhoea, deranged transaminases, and hypothyroidism were the most commonly reported. Looking specifically at the immunotherapy-naïve group, overall response rate was 27–30% (depending on the criteria used). The complete response in both criteria was 10% [47]. There are ongoing phase 3 studies (KEYNOTE-252/ECHO-301) investigating pembrolizumab and epacadostat in advanced melanoma. These trials will likely have a significant impact on the treatment algorithms for advanced melanoma.

3.1.4. Combination therapy

CTLA4 inhibitors and anti-PD1 inhibitors have been recently combined to determine if combination therapy offers improved efficacy vs. monotherapy, with clinically acceptable safety outcomes. Checkmate 067 was a phase 3, multicentre trial which included patients with previously untreated stage III (unresectable)/IV melanoma with known BRAFV600 status. A total of 945 patients underwent randomisation to receive either nivolumab/ipilimumab or ipilimumab alone. All living patients had a minimum follow-up of 36 months, with a median follow-up of 38 months. The overall survival rate at 3 years was 58% in the nivolumab/ipilimumab vs. 34% in the ipilimumab group. Treatment-related adverse events of grade 3/4 were reported in 59% participants in the nivolumab/ipilimumab group, in 21% of the nivolumab group and 28% of the ipilimumab group. 32%, 46%, and 63% of patients received subsequent systemic therapy in the nivolumab/ipilimumab, nivolumab and ipilimumab group, respectively.

There were two deaths related to a study drug within 100 days and two deaths related to a study drug more than 100 days [48].

3.2. Autologous ex-vivo augmented tumour infiltrating lymphocytes

The immune response can be increased by either in vivo vaccination or proliferation of the antigen-specific effectors in vitro followed by transfer to the patient. The APCs used for generating effector responses are critical for determining the specificity and type of immune response. However, the response and essentially the outcome of the T-cells differ hugely whether it is in vivo or in vitro [49].

Adoptive cell therapy, unlike checkpoint inhibitors, creates an immune response, rather than simply “taking the breaks” off the immune system. It involves harvesting the T cells from the patient’s serum or tumour and then encouraging them to proliferate in a culture medium in vitro [25]. Adoptive cell therapy involves lymphodepletion prior to infusing autologous tumour infiltrating lymphocytes. In three clinical trials involving 93 patients with refractory melanoma, the response rate varied from 48 and 72%, depending on the chemoradiation strategy employed for the lymphodepletion technique. The median follow-up varied between 10 and 45 months for the trials. The 2-year survival rates ranged from 30 to 42%. There was one treatment-related death [50]. This treatment strategy only experimental and has not been approved by the US FDA for the treatment of melanoma.

3.3. Oncolytic viruses

An oncolytic virus is a non-pathogenic virus which destroys cancer cells, while leaving the normal cells unaffected [51]. The use of oncolytic viruses offers an attractive treatment strategy. The oncolytic viruses result in cytotoxic effects by directly infecting the cancer cells. Furthermore, the viral genome can be manipulated to maximise the beneficial therapeutic effects and to minimise harmful effects. Oncolytic viruses involve the administration of either native or genetically modified viruses, which then enter the tumour cell selectively, proliferate and lyse these cells [52]. The endogenous defence mechanisms against viral-mediated infection are suboptimal in tumour cells. This results in a high turnover of the virus in the tumour cell. The cancer-specific replication is achieved by either selecting a non-virulent virus in humans or by manipulating the genome of the virus [53]. The viruses succeed in destroying the tumour cells through several mechanisms: primary lysis of the cancer cells, powerful bystander effects on healthy cells, provocation of local endogenous antiviral mechanisms and systemic antitumor immunity that can cause regression of the cancer at distant, uninfected cells. There are several types of oncolytic viruses developed: oncolytic poxvirus, oncolytic herpes simplex virus, oncolytic Coxsackie virus and oncolytic reovirus [52].

There are several barriers to treatment with oncolytic viruses: pathogenic potential, suboptimal ability to selectively target cancer cells, degradation by the immune system, and suboptimal ability to trigger T-cell response to neoplasm. Despite these limitations, the first oncolytic therapy known as laherpaprepvec was approved by the FDA for melanoma patients in 2015. It was indicated for patients with injectable lesions in the skin and lymph nodes that were not amenable to surgical resection [51].

Laherparepvec is a genetically engineered oncolytic virus. It is a genetically modified HSV-1. Laherparepvec is used for the treatment of melanoma. It works in two different ways: it replicates more actively in the tumour cells. This causes lysis of the tumour cells. Viral particles and tumour-associated antigens are released from the cells. The viral cells can preferentially target more tumour cells. The tumour antigen can induce an immune response, which is potentiated by the expression of GM-CSF in the laherparepvec. Laherparepvec functions by exploiting the protein kinase R (PKR) pathway. This pathway suppresses viral replication in healthy cells. The usual defence mechanism infected susceptible cell protein 34.5 is usually responsible for overcoming the PKR pathway in HSV. However, laherparepvec is genetically modified to delete the infected susceptible cell protein 34.5 in HSV-1, leaving the cells vulnerable to degradation by PKR. In healthy cells, the PKR pathway is active and causes inactivation of the laherparepvec pathway. However, in the case of tumour cells, PKR is inactive. This leads to the virus actively replicating selectively in the tumour cells. Furthermore, there is a downregulation of type 1 IFN pathway in tumour cells. This leads to a further susceptibility of tumour cells to laherparepvec [54]. The OPTiM trial randomised patients to either intralesional laherparepvec or subcutaneous GM-CSF in patients with stage 3 and 4 melanomas. The trial showed monotherapy with laherparepvec significantly increases the durable response rate vs. therapy with GM-CSF alone (25.2% vs. 1.2%, respectively). It also improved the overall response rate (40.5% vs. 2.3%, respectively). The toxicity profile was similar in both treatment arms, with the majority of toxicities including grade 1 and 2 toxicities [55]. There are ongoing phase 3 trials examining the efficacy of combination therapy with laherparepvec and pembrolizumab in stage 3 and 4 melanomas. Earlier phase 2 trials appear promising.

3.4. Dendritic cells

Dendritic cells are a form of immune cell, which is the more powerful antigen-presenting cell. The cells circulate in their inactive state in the body circulation. When they are exposed to a danger signal, they become activated antigen-presenting cells. They facilitate immune responses in the lymphoid tissue, causing the naïve T-cells to differentiate into effector T cells. DC cells facilitate activation of tumour immunity. They activate antigen-specific T cell responses in melanoma patients. Dendritic cell vaccines are activated dendritic cells containing tumour antigens. Dendritic cells are not advisable as monotherapy in the treatment of advanced melanoma. However, there are promising results when DC viruses are combined with ipilimumab. It is postulated that the immune system is more potent in stage 3 vs. stage 4 melanoma. Dendritic cells show some promise in stage III melanoma. However, phase 3 trials are pending. The safety profile of DC vaccines is favourable when compared with checkpoint inhibitors [56].

4. Chemotherapy

In the advent of targeted therapies, chemotherapy is no longer deemed a first-line therapy for metastatic cutaneous melanoma in the latest ESMO guidelines [2]. However, in the recent past, chemotherapy was an important therapeutic strategy for palliation. Examples of chemotherapeutic agents employed in melanoma include dacarbazine, temozolomide, nab-paclitaxel, paclitaxel, cisplatin, carboplatin, and vinblastine. The only chemotherapy agent approved by the FDA

is dacarbazine. Dacarbazine and temozolomide (an analogue drug) are alkylating agents that damage DNA, leading to cell apoptosis. Multiple phase 3 studies have failed to demonstrate an overall survival benefit for any chemotherapy regimen. Specifically, only 10–20% will respond to dacarbazine. The progression-free rate is 3–6 months with dacarbazine. The adverse effects of dacarbazine include bone marrow suppression and nausea/vomiting. Former combination regimens included BOLD (bleomycin, vincristine, lomustine and dacarbazine), CVD (cisplatin, vinblastine and dacarbazine) and the Dartmouth regimen (dacarbazine, cisplatin, carmustine and tamoxifen). However, the studies failed to demonstrate a benefit to combination chemotherapy vs. monotherapy. Furthermore, the toxicity profiles of the combination therapy were worse than monotherapy. Combining immunotherapy and chemotherapy similarly failed to demonstrate any significant benefit. It also led to worse outcomes in terms of toxicity profiles [57].

5. Conclusion

In conclusion, immunotherapy and targeted therapy in the form of BRAF/MEK inhibitors form the backbone of therapy for metastatic melanoma. The optimal agents remain under considerable debate. Chemotherapy has been relegated to second-line therapy. Future guidelines will likely reflect this new research.

In conclusion, the mainstay treatment for managing melanoma remains surgery if feasible. There are several adjuvant therapies such as anti-PD1 therapies, CTLA4 inhibitors and BRAF/MEK inhibitors that may play a useful role as adjuvant therapies in high-risk, stage 3 disease. The treatment strategies for advanced melanoma are evolving rapidly. Targeted therapies such as anti-PD1 therapies, CTLA4 inhibitors and BRAF/MEK inhibitors have become mainstay treatment. Further research must be carried out to determine the best regimen. Chemotherapy now only plays a role in rescue therapy.

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