The role of immunotherapy and molecular-targeted therapy in the treatment of melanoma (Review)

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Abstract. Skin melanomas are malignant neoplasms originating from neuroectodermal melanocytes. Compared to other neoplasms, melanomas have a high rate of growth. Their incidence is highest in Australia and New Zealand, in high-income European countries (Switzerland, Norway, Sweden) and in the US. In Poland, the standardized incidence rate is approximately 5/100,000. Melanomas are typically highly radioresistant and chemoresistant. Before the era of immunotherapy, inoperable lesions were treated using chemotherapy based mainly on dacarbazine, temozolomide or fotemustine, which did not yield the expected results in terms of extending survival time or improving patient comfort. Therefore, there has emerged a need to seek other solutions. In most cases, the use of immunological treatment or targeted therapy has had a positive impact on survival time and relapse-free survival. However, these periods are still relatively short, hence the need for further research and improvement of treatment. The most promising strategies appear to be antibodies that block programmed death receptor-1 (PD-1) and programmed death receptor ligand-1 (PD-L1) molecules, anti-CTLA4 antibodies (cytotoxic T-lymphocyte antigen 4) and therapy with BRAF and MEK inhibitors.

Contents

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1. Introduction

Skin melanomas are malignant neoplasms derived from neuroectodermal melanocytes. Although they can develop on pre-existing melanocytic nevi, in more than 50% of cases they arise *de novo* (1). There are three subtypes of melanoma: Cutaneous (the most common), mucosal and ocular (in most cases choroidal), the latter being the most common extracutaneous melanoma. Compared to other neoplasms, melanomas have the highest rate growth. There are over 100,000 new cases every year, with the highest incidences found in Australia and New Zealand, high-income European countries (Switzerland, Norway, Sweden) and in the US (1). In Poland, the standardized incidence rate is of the order of 5/100,000, which corresponds to approximately 3,100 cases per year (2). Skin melanoma accounts for approximately 2% of all cancers diagnosed in Polish men and women (3). The incidence of skin melanoma is greater after the age of 20, with the highest incidence in both sexes between 50 and 64 years of age. The risk of melanoma increases with age, reaching its maximum in the eighth decade of life. Skin melanoma causes about 1.4% of deaths due to cancer in men and 1.5% in women. The standardized mortality rate in Poland reaches 2.3/100,000 men and 1.5/100,000 women (2). The relative 5-year survival rate for stage IV melanoma in Poland is 5-10%, whereas in Western Europe and the US this rate reach 28%, which is probably related to earlier detection, and thus a less advanced stage at diagnosis (3-5). The outcomes of cancer treatment in Poland are worse than in most European countries. The largest differences in effectiveness of treatment occur in the case of melanoma (Germany 93.1 vs. Poland 69.8) (3).

The most significant factors in an increased risk of malignant melanoma include: Ultraviolet radiation, mechanical or chemical irritation, low content of pigment in the skin, previous melanoma and genetic predisposition, including familial atypical multiple mole melanoma syndrome (1). At the time of diagnosis, skin melanoma in approximately 80% of patients is present in the form of a local lesion, while in approximately 15% it is locally advanced and metastatic in 5%. The location in the integument makes early identification of a lesion possible, which is conducive to curing the tumor completely using surgical methods (6).

Surgical treatment remains the main method of melanoma treatment (7). It consists in complete excision of the scar

after an excisional biopsy performed at the diagnostic stage (micro-staging I) with a sufficiently large margin of healthy tissue, which depends on the infiltration depth of the lesion. To date, no increase in survival has been confirmed for margins of more than 2 mm around excised lesions with an infiltration depth greater than 2 cm (8).

A sentinel node biopsy (micro-staging II) is performed concurrently with the widening of the resection margins. Sentinel lymph node biopsy (SLNB) should be considered in all patients in stage IB or II due to the fact that the presence of metastases in local lymph nodes is the most significant prognostic factor in patients with cutaneous melanoma (7). Research has confirmed the importance of SLNB in cancer staging and in the identification of patients with lymph node metastases whose survival time may be extended by selective lymphadenectomy (9,10). However, it has been shown that it only affects relapse-free survival, not overall survival. In the case of patients with no lymph node metastases, the overall survival rate is approximately 90% (9). Routine elective lymphadenectomy is not recommended (11).

Melanomas are typically highly radioresistant and chemoresistant. Before the era of immunotherapy, inoperable lesions were treated with chemotherapy based mainly on dacarbazine, temozolomide or fotemustine. It has been shown that the immune system and mechanisms play an important role in oncology and that new principles of immunotherapy are also used for melanoma because it is a form of cancer suitable for treament with immunotherapy (12). Qualification for novel therapies should be preceded by testing for the presence of certain changes in cell metabolism or alterations of the genetic profile which can be targeted during treatment. It is crucial for directing patients to the appropriate targeted treatment or clinical trial. In the case of melanoma, tumor tissues should be screened for mutations of BRAF V600 to identify patients who may benefit from treatment with BRAF or MEK inhibitors. If the results are negative, further molecular testing can be carried out for NRAS or c-Kit (11). The influence of programmed death receptor-1 (PD-1) expression on treatment with anti-PD-L1 is still debated and is not unambiguous (11). Unfortunately, responses to targeted therapy are generally not complete or long-lasting as resistance mechanisms develop upon continuous drug exposure. Combination of different types of molecular-targeted therapy agents co-inhibiting two or more targets in single or complementary pathways may improve treatment efficacy (11). The most common melanoma treatment is a combination of BRAF and MEK inhibitors.

2. Immunotherapy

Cytokines. Interferon (IFN)-α is a cytokine produced primarily by plasmacytoid dendritic cells as a result of stimulation of their TLR7 and TLR9 receptors (Toll-like receptors) by cytokine stimulation [interleukin (IL)-1, IL-2, tumor necrosis factor (TNF)-α] and viral infection (13). Binding of IFN-α to the receptor, JAK tyrosine kinase (Janus-activated kinase) is activated, which results in an increase in immune system responses, inhibition of cell proliferation and stimulation of their differentiation (14). Recombinant IFN-α-2b (Intron A) was the first agent shown to significantly improve survival in a phase III randomized trial in which survival time was prolonged by about one year in adjuvant therapy in patients treated with the drug in comparison to the observed group (15). As early as 1995, IFN-α-2b has been registered by the FDA (Food and Drug Administration) for adjuvant treatment of melanoma in patients at high risk of relapse (15). Alfa-Peginterferon-2b, an interferon derivative, was created by coupling with polyethylene glycol and has been approved by the FDA for adjuvant therapy in 2011 on the basis of a study showing prolongation of relapse-free survival compared to non-treated patients (34. 8 months vs. 25.5 months) (16). Cytokines can act through an increase in natural killer (NK) cell activity (17,18). Side effects related to the ingestion of interferons usually include flu-like symptoms.

IL-2 is a cytokine secreted primarily by T helper 1 (Th1) lymphocytes that recognise antigens (19). The IL-2 receptor couples to JAK tyrosine kinases and activates the signal transducer and activator of transcription 5 (STAT5) (16). It is mainly responsible for the regulation of lymphocyte activity and protection against autoimmunization (19). It was approved by the FDA in 1998 for the treatment of stage IV patients. The median survival time in 8 different clinical trials was 11.4 months. If complete responses to treatment were obtained, they were long; even up to 40 months (20). A pooled analysis showed a complete response to IL-2 administration to in-transit lesions in 50% of subjects and excellent tolerability of treatment in 78% (21).

Anti-CTLA4 antibodies, (CD152). Cytotoxic T-lymphocyte antigen 4 (CTLA-4) is a receptor found on regulatory T lymphocytes and on activated $CD4^+$ and $CD8^+$ T lymphocytes (22). There are two forms of the CTLA-4 receptor: flCTLA-4, which is anchored to the cell membrane, and sCTLA-4, which is soluble in serum (23).

The ligands for this receptor are B7.1 (CD80) and B7.2 (CD86) molecules present on antigen-presenting cells (APCs), which are also ligands for the CD28 molecule (24). The binding of CD28 to CD86 or CD80 enhances the secretion of IL-2 and the proliferation of T lymphocytes (24). CTLA-4 has a higher affinity for ligands than CD28. Binding of the CTLA-4 receptor to a B7 ligand inhibits activation and proliferation as well as depression of the effector functions of T lymphocytes, thus constituting an element of negative feedback of the immune response (25). This results in inhibition of the immune system responses and encouragement of neoplastic processes. The use of antibodies blocking the CTLA-4 receptor prevents its binding to a ligand, thereby increasing the activation of lymphocytes and the antitumor immune response.

Ipilimumab (trade name Yervoy) is a human IgG1k monoclonal antibody targeting CTLA-4. It is the first drug shown to prolong survival and periods of remission in metastatic melanoma. In 2011, it was approved by the FDA for the treatment of disseminated melanoma based on a phase III study (26), and in 2015 for adjuvant treatment (27). The registration trial for adjuvant treatment was a phase III trial where the remission period in the drug group was approximately 26 months on average, compared to 17 months in the placebo group (27). The use of ipilimumab is also associated with prolonged survival (26-28). In a single-arm phase II trial of pretreated metastatic malignant melanoma, the median overall survival was about 10 months (28). Phase I trials have also shown a

response to ipilimumab administered with IL-2 to the tumor site resulting in a reduction in the injected primary in 67% of subjects and the local metastatic lesions in 89% (29). Its efficacy has been shown to be higher for the dose of 10 mg/kg of body weight than for 3 mg/kg of body weight (30).

Another drug in this group is tremelimumab (human monoclonal antibody against CTLA-4). A phase III study on disseminated melanoma did not show that it had any significant advantage over chemotherapeutics (dacarbazine, temozolomide). The median survival in patients receiving tremelimumab was 12.6 months, compared to 10.7 months in patients receiving cytostatics (31). Tremelimumab has not been approved by the FDA.

The use of CTLA-4 antibodies is associated with significant adverse autoimmune effects such as dermatitis, endophthalmitis, colitis and diarrhea (32).

Molecules blocking the PD-1 receptor (programmed death receptor-1). The PD-1 receptor of the CD28 family is found on $CD4^+$ and $CD8^+$ T lymphocytes, B lymphocytes, NK cells, monocytes and activated dendritic cells (33,34). Like CTLA-4, it is responsible for inhibiting antitumor responses. It has two ligands: Programmed death-ligand 1 (PD-L1) found in many different tissues and the less widespread programmed death-ligand 2 (PD-L2) present on specialized antigen-presenting cells (35). The combination of the PD-1 receptor with either PD-L1 or PD-L2 results in suppression of the immune system, for example by reducing the production of cytokines and increasing the synthesis of IL-10 which inhibits the immune response (36,37). Activation of the PD-1/PD-L1/PD-L2 pathway allows cancer cells to evade the immune system response through negative regulation of effector T lymphocytes (Fig. 1). PD-L1 expression has been observed in the case of lung, prostate and kidney cancer, as well as in melanoma (38-40). The mechanism responsible for modulation of PD-L1 expression by cancer cells has not yet been identified. Currently, most drugs in this group block the PD-1 receptor.

Pembrolizumab is a humanized monoclonal IgG4 antibody targeting the PD-1 receptor and blocking its interaction with PD-L1 and PD-L2 ligands. It has higher efficacy and better tolerance in patients in advanced stages, compared to ipilimumab [response rate (RR), 33 vs. 12%] (41). In 2014, it was approved by the FDA for the treatment of metastatic melanoma based on a phase Ib trial (42), becoming the first registered PD-1 inhibitor. In subsequent years, its indications have been extended to include the treatment of head, neck and lung cancer. In 2019 the FDA approved pembrolizumab for the adjuvant treatment of patients with melanoma with involvement of lymph node(s) following complete resection. The approval was based on a randomized, double-blind, placebo-controlled, trial which demonstrated that pembolizumab provided a clinically meaningful sustained improvement in recurrence-free survival in resected high-risk stage III melanoma (43).

Another drug in this group is nivolumab, a human IgG4 antibody targeting the PD-1 receptor, which is used in the treatment of kidney, liver and lung cancer. In 2014, the FDA approved the use of nivolumab in patients with inoperable metastatic melanoma with a mutation resulting in the substitution of valine at position 600 with another amino acid in the BRAF protein (V600) (44). In 2015, the combination of

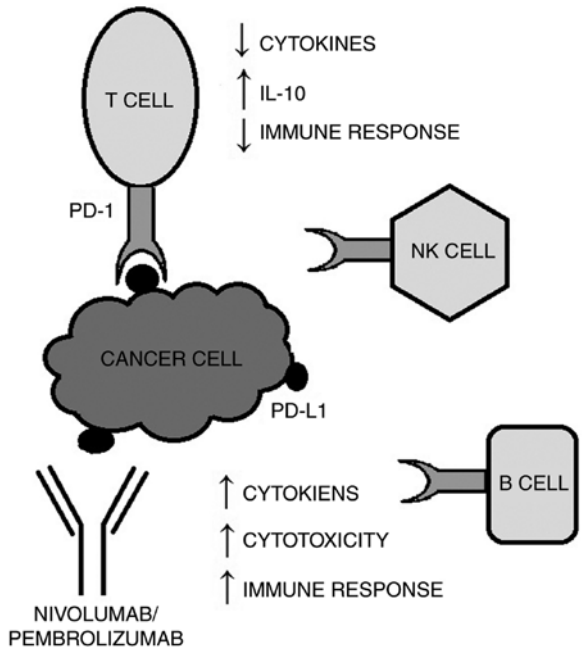

Figure 1. Mechanism of action of PD-1 inhibitors. The PD-1 receptor is expressed among others on activated T cells, B cells, and natural killer (NK) cells. Activation of PD-1/PD-L1 signaling serves as a principal mechanism by which tumors evade antigen-specific T-cell immunologic responses. Antibody blockade of PD-1 reverses this process and enhances antitumor immune activity. PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1.

nivolumab and ipilimumab was approved on the basis of a phase II trial (45). In a randomized phase III trial, the efficacy of adjuvant treatment of patients in stages IIIB, IIIC and IV with nivolumab was shown to be higher than with ipilimumab. It also results in a longer progression-free survival (PFS) and less frequent occurrence of severe side effects in comparison to ipilimumab. The trial involved 906 patients, half of whom received nivolumab at a dose of 3 mg/kg of body weight every 2 weeks, while the others received ipilimumab at a dose of 10 mg/kg of body weight every 3 weeks, then every 12 weeks, for about a year. The 12-month relapse-free survival rate for nivolumab was 70.5%, and for ipilimumab it was 60.8% (46). This study became the basis for the FDA approval of nivolumab for the adjuvant treatment of patients with advanced melanoma in 2017 (47).

Research shows that antitumor effects may be induced not only by blocking the PD-1 receptor, as blocking PD-L1 on dendritic cells also leads to an increase in the activity and function of effector lymphocytes and slowing tumor growth (48). In a phase I clinical trial on the BMS-936559 antibody targeting PD-L1, a response was obtained in 9 out of 52 patients with advanced melanoma (49).

Because of the activation of the immune system, PD-1 and PD-L1 inhibitors can cause a specific set of inflammatory side effects, known as immune-related adverse events (irAEs) (50). irAEs can occur in any organ system and can be categorized into five ascending grades of symptoms: Asymptomatic/mild (grade 1), moderate (grade 2), severe (grade 3), life-threatening (grade 4), and death (grade 5) (51). Anti-PD-1 agent toxicities appear to be dose-independent and the overall incidence of severe or life-threatening irAEs (grade ≥ 3) ranges from 10 to 15% for patients during this type of treatment (51). The

organs with the highest reported irAE incidences are skin, gastrointestinal tract, liver and lungs (51). Although mild irAEs can generally be treated supportively, severe toxicity may be fatal and requires urgent intervention (51). The most common irAEs are dermatological toxicities, which usually appear around 3 to 6 weeks after therapy initiation (51).

3. Targeted treatment

RAS/RAF/MAPK pathway disorders and drugs blocking its activity. The RAS/RAF/MAPK (mitogen-activated protein kinase) signaling pathway transmitting the signal from the cell surface to the cell nucleus is responsible for the control of cell proliferation, differentiation, migration and survival. After a signal molecule attaches to the receptor in the cell membrane, the RAS protein (product of the *RAS* proto-oncogene-rat sarcoma viral oncogene homolog) is stimulated and becomes an active kinase through phosphorylation, activating another protein of the pathway, the RAF protein with serine/threonine kinase activity. The RAF protein has 3 isoforms: ARAF, BRAF and CRAF, with BRAF being the strongest activator of MEK kinase (mitogen/extracellular signal-regulated kinase). The active BRAF kinase phosphorylates MEK1 and MEK2 proteins that activate extracellular signal-regulated kinase (ERK)1 and ERK2 proteins. The activated ERKs transmit the signal to the cell nucleus, which induces the expression of genes responsible for cell growth and survival (52,53). In melanoma cells, hyperactivity of the RAS/RAF/MEK/ERK(MAPK) pathway is observed. During the development of melanoma, its autocrine and paracrine cells secrete growth factors, including transforming growth factor (TGF)-β, IL-6 and IL-8, vascular endothelial growth factor (VEGF), and platelet-derived growth factor subunit A(PDGF-A) (54), which cause constitutive activation of the RAS/RAF/MAPK pathway. The most frequent cause of hyperactivity of this pathway is a BRAF kinase mutation, which is observed in approximately 50% of advanced melanomas (55). The main point mutations are missense mutations where valine (V) is substituted at position 600 with glutamic acid (E) or lysine (K) (V600E, V600K). V600E mutations (around 80%) occur most often in young people, whereas V600K mutations (around 20%) in the elderly. Both mutations determine a more aggressive course of advanced melanoma (56). Patients with BRAF mutations are more likely to present with metastases to local lymph nodes (57) (Fig. 2).

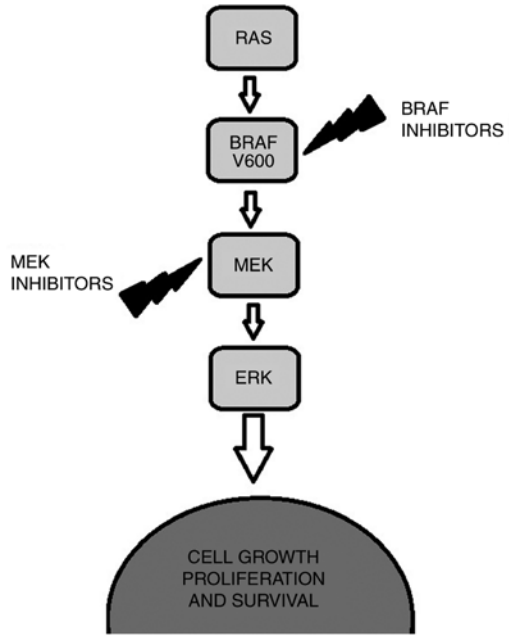

Figure 2. Mechanism of action of BRAF and MEK inhibitors. In melanoma cells, hyperactivity of the RAS/RAF/MEK/ERK(MAPK) pathway is observed leading to extensive cancer cell proliferation and enhanced survival. The most frequent cause of hyperactivity of this pathway is a BRAF kinase mutation, V600E mutations (around 80%) occur most often. Inhibition of mutant BRAF (by BRAF inhibitors: vemurafenib, dabrafenib) or MEK (by MEK inhibitor: Cobimetinib, binimetinib) shuts down the ERK signaling thus inhibits cell proliferation. BRAF, B-Raf proto-oncogene, serine/threonine kinase; MEK, mitogen-activated protein kinase.

BRAF inhibitors. Sorafenib is a broad-spectrum inhibitor. It inhibits the activity of many RAF serine/threonine kinases (CRAF, BRAF, V600E BRAF) and membrane receptor tyrosine kinases such as: Mast/stem cell growth factor receptor (CD117), FMS-like tyrosine kinase 3 (FLT3), vascular endothelial growth factor receptors (VEGFRs), and platelet-derived growth factor receptors (PDGRs). Despite FDA approval for the treatment of kidney, liver and thyroid cancer, the results of studies concerning sorafenib in melanoma treatment were unsatisfactory. It was found to have little or no activity either in monotherapy (median PFS was 11 weeks) (58) or in combination with chemotherapy (the RR for carboplatin+paclitaxel+sorafenib vs. carboplatin+paclitaxel+placebo was 12% vs. 11%) (59).

Selective inhibitors include vemurafenib and dabrafenib, which block BRAF activity. A phase III clinical trial on a group of 675 previously untreated patients with disseminated melanoma with a BRAF V600E mutation showed a 74% reduction in risk of progression and a 63% reduction in risk of death in the vemurafenib group, compared to the dacarbazine group. The RR was 48% for vemurafenib and only 5% for dacarbazine (60). Based on the results of this study, vemurafenib became the first molecular-targeted drug registered in the European Union and the US for the treatment of patients with advanced disseminated melanoma and a confirmed BRAF V600 mutation (61). Dabrafenib had equally positive results. In a phase III trial, the median PFS was approximately 5 months, compared to about 2 months for dacarbazine, with a worse response in patients with a V600K mutation, compared to a V600E mutation (62). This study became the basis for the approval of dabrafenib by the FDA in 2013.

Oral administration of both vemurafenib and dabrafenib has been associated with adverse effects. In the case of the first drug, they included joint pain, rash, nausea and diarrhea, hypersensitivity to ultraviolet (UV) radiation, hair loss and proliferation of keratinocytes (in about 20% of subjects); in the case of the second drug, adverse effects consisted of fever, skin lesions, headaches and joint pain. Their occurrence entailed the need to reduce the doses in both cases by approximately 30% (60,62,63).

MEK inhibitors (mitogen-activated protein kinase kinase, MAP2K). One of the drugs in this group is binimetinib. It was shown to have greater benefits in the treatment of

metastatic melanoma, compared to dacarbazine (increase in PFS: 2.8 months vs. 1.5 months) (64). Another drug in this group is selumetinib, an inhibitor of MEK1 and MEK2. In a phase II study concerning selumetinib, there was no significant difference found in PFS compared to treatment with temozolomide (78 days vs. 80 days) (65). Similar results were obtained in phase II studies in which docetaxel was administered either alone or in combination with selumetinib (PFS: 3.9 months vs. 4.2 months) (66). The FDA has not approved treatment of disseminated melanoma with either binimetinib in monotherapy or selumetinib. The activity of trametinib, a selective inhibitor of MEK1 and MEK2, has been confirmed both in patients with a BRAF mutation and without it (wild-type BRAF). In a phase III study, in which patients with a V600 mutation were randomly assigned to either chemotherapy or trametinib group, it was shown that treatment with the inhibitor prolongs median PFS by just over 3 months, compared to monotherapy with a chemotherapeutic agent. After 6 months of treatment, a higher survival rate was found in the trametinib group, compared to the group treated with chemotherapy (81 and 67%, respectively). Based on this study, the drug was approved by the FDA in 2013 for the treatment of melanoma, becoming the first commercially available MEK inhibitor. The most serious adverse effects observed during the study were reduction of the heart's ejection fraction and impaired vision, whereas the most common ones were diarrhea, rash and limb edema (67).

Cobimetinib is another selective MEK inhibitor. It was registered by the FDA in 2015 for the treatment of metastatic melanoma with a BRAF V600E or V600K mutation in combination with vemurafenib. In a phase III registration trial, the group receiving cobimetinib in combination with vemurafenib showed an increase in PFS, compared to the vemurafenib group (9.9 months vs. 6.2 months) (68).

Combination of BRAF and MEK inhibitors. Due to the resistance that develops during treatment with BRAF inhibitors, combination therapies with MEK inhibitors have been attempted. A phase III study concerning a dabrafenib/trametinib combination administered to previously untreated patients with metastatic melanoma showed higher median PFS for combination therapy than for dabrafenib monotherapy (9.3 months vs. 8.8 months), with RR of 67 and 51%, respectively (69). In another study, in which patients received either dabrafenib with trametinib or only vemurafenib, the median PFS was 11.4 and 7.3 months, respectively, with RR of 64% vs. 51% (70). Studies involving patients with a good initial response to the BRAF inhibitor and a subsequent worsening of the treatment results found that combination therapy with these drugs had low efficacy (71,72). In patients treated with a combination of the two drugs, systemic adverse effects were more frequently observed, whereas patients treated with dabrafenib monotherapy were more likely to develop hyperkeratosis (72). In 2018, the FDA granted regular approval to dabrafenib and trametinib in combination for the adjuvant treatment of patients with melanoma with BRAF mutations and involvement of lymph nodes following complete resection. The approval was based on an international, multi-center, randomized, double-blind, placebo-controlled trial in 870 patients with stage III melanoma with BRAF V600E or V600K mutations, and pathologic involvement of regional lymph nodes. Patients who received the combination treatment had a statistically significant improvement in RFS (relapse-free survival) compared with those receiving placebo (73).

Recent studies have investigated new combinations of BRAF and MEK inhibitors. The latest published study results concern encorafenib (a BRAF kinase inhibitor) which has been shown to be more effective than vemurafenib for the treatment of metastatic patients, and its combination with binimetinib (a MEK inhibitor) has an even more beneficial effect on RR (median PFS for drug combination vs. encorafenib monotherapy vs. vemurafenib monotherapy is 14.9 months vs. 9.6 months vs. 7.3 months, respectively) (74). Consequently, the FDA issued a decision in June 2018 to approve the combination of encorafenib and binimetinib for treatment of metastatic melanoma with a BRAF V600E or V600K mutation.

Farnesyltransferase inhibitors. Farnesyltransferase is a cytosolic enzyme responsible for the transfer of the farnesyl group of farnesyl diphosphate to the CAAX motif (C, cysteine; A, aliphatic residue; X, any amino acid) of the RAS protein, which facilitates its attachment to the internal membrane of a plasma cell and allows initiation of transmission (75). The inhibition of this process is a method of blocking RAS activity.

The only farnesyltransferase inhibitor investigated to date is tipifarnib, which is characterized by significant toxicity. Moreover, its antitumor activity has not yet been confirmed (76).

Genetic disorders of the PI3K/AKT/mTOR pathway. The phosphatidylinositol 3-kinase/serine/threonine kinase/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway is an intracellular signalling pathway that can be activated in a variety of ways, including growth factors: Epidermal growth factor receptor (EGFR) or insulin-like growth factor-1 receptor (IGF-1R). Activation of phosphatidylinositol 3-kinase (PI3K kinase) leads to increased production of phosphatidylinositol (3-5)-trisphosphate (PIP3), which in turn leads to increased recruitment of serine/threonine kinase-protein kinase B (AKT/PKB) to the cell membrane and its activation (77). The AKT kinase family includes 3 proteins: AKT-1, AKT-2, AKT-3 (72). Active AKT kinase regulates the activity of the Bcl-2-associated death promoter (BAD) protein, the nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) protein and the serine/threonine kinase mammalian target of rapamycin (mTOR), thus regulating the processes of apoptosis, angiogenesis and proliferation. This pathway is controlled by the phosphatase and tensin homolog (PTEN) phosphatase, whose mutation or expression disorders are quite often found in melanomas. Activation of this pathway in cancer cells results in a decrease in apoptosis and an increase in proliferation (77).

Inhibitors of the PI3K/AKT/mTOR pathway. One of the first drugs in this group subjected to trials was perifosine (an inhibitor of AKT and PI3K). In phase II trials, it was characterized by good tolerance of treatment, but no objective response was observed in 14 subjects, and 11 of them showed disease progression (78). In a phase II trial of another drug, temsirolimus (an inhibitor of mTOR kinase), which

was successfully used to treat kidney cancer, only 1 out of 33 patients responded to the treatment, with a partial remission lasting 2 months (79). Phase II trials concerning everolimus (an mTOR kinase inhibitor), which also belongs to this group, did not find sufficient activity of this drug or any significant impact on PFS either (2 months on average) (80).

c-Kit inhibitors (CD117-receptor tyrosine kinase). The c-KIT receptor is a surface receptor with tyrosine kinase activity. It is activated by binding a ligand, which is a stem cell growth factor (SCF). Activation of the c-KIT receptor by SCF leads to the activation of cell signaling that regulates the processes of cell migration, survival, proliferation and differentiation (81,82). The ultimate effect is uncontrolled growth and proliferation of cancer cells, inhibition of apoptosis, promotion of angiogenesis and metastasis (83). Tyrosine kinase inhibitors bind and block the domain responsible for the binding of ATP (adenosine-5'-triphosphate). This combination prevents activation of the kinase and, consequently, a cascade of proteins transmitting a proliferative signal to the cell nucleus. Most of the inhibitors are non-specific and have affinity for several tyrosine kinases (84). It is currently believed that c-KIT mutations in melanoma are rare and occur only in some of its subtypes.

Imatinib is an antibody that acts by, for example, competitively blocking the kinase activity resulting from the fusion of the BCR gene (breakpoint cluster region) and the ABL gene (ABL1-Abelson murine leukaemia viral oncogene homolog 1). It is also an inhibitor of receptor tyrosine kinases of platelet-derived growth factor (PDGF) and SCF. In two independent phase II clinical trials conducted on patients with metastatic melanoma, the efficacy of treatment with imatinib was found to be low. In the 2008 study, the median time to progression was 1.4 months and the median total survival time was 7.5 months (85). The median PFS in the 2011 study was 3.5 months (86). Another drug in this group was nilotinib, whose mechanism of action is similar to imatinib. In a phase II study, its activity was comparable to imatinib, with the objective response rate at 26.2% and a slightly higher activity in subjects with a mutation in exon 11. The median PFS was 4.2 months, and the total survival time was 18 months (87). Dasatinib, another drug in this group, inhibits the activity of c-KIT and BCR-ABL kinases as well as the SRC family of kinases. However, no significant activity of this drug was demonstrated in a phase II study. The median PFS was 8 weeks, but the toxicity of treatment was significant and a reduction in the doses was required (88).

TRK inhibitors. The tropomyosin receptor kinase (TRK) is a family of three transmembrane receptor tyrosine kinases (TRKA, TRKB, TRKC) which are encoded respectively by neurotrophic receptor tyrosine kinase 1 (*NTRK1), NTRK2, NTRK3* genes and have a role in the development and normal functioning of the nervous system (89). The TRK receptors are activated by four different neurotrophins (89). Nerve growth factor (NGF) has affinity for TRKA (84), brain-derived neurotrophic factor (BDNF) and neurotrophin 4 (NT-4) bind to TRKB and neurotrophin 3 (NT-3) has affinity for TRKC (89). Phosphorylation is required for activation of the TRK receptor and is preceded by neurotrophin binding to TRK receptors at the cell surface which causes the formation of receptor dimmers. The binding of TRKA by NGF causes activation of the RAS/MAPK pathway which results in increased cellular proliferation and growth via ERK signaling (90). Activation of TRKB leads to activation of the RAS-ERK, PI3K and PLCγ pathway, resulting in neuronal differentiation and survival (90). Activation of TRKC leads to activation of the PI3K/AKT pathway, preventing apoptosis and increasing cell survival (90). Fusions involving the *NTRK* gene family (*NTRK1, NTRK2, and NTRK3*) lead to the expression of chimeric rearrangements in TRKA, TRKB, and TRKC, respectively, with constitutively active kinase function, promoting cell proliferation and survival (91). In the *NTRK* gene fusion the 3' region of the *NTRK* gene is joined with the 5' end of a fusion partner gene (91). *NTRK* gene fusions have been estimated to occur predominantly in less than 1% of all solid tumors and less than 5% of all melanomas (92). To date, there are two TRK inhibitors approved for treatment of *NTRK* fusion-positive melanoma.

Larotrectinib is a potent and highly selective small-molecule inhibitor of all three TRK proteins (93). In 2018, larotrectinib was approved by the FDA for the treatment of adult and pediatric patients with solid tumors that harbor a *NTRK* gene fusion. The FDA based its approval on 3 clinical trials that included 55 adults and children with several different cancer types, 4 of whom had melanoma (94). The overall RR was 75% with uncommon clinically significant adverse events (94).

Entrectinib is a potent oral inhibitor of the tyrosine kinases TRKA/B/C, as well as ROS proto-oncogene 1 (ROS1) and anaplastic lymphoma kinase (ALK) (94). The robust antitumor activity of entrectinib has been demonstrated in three phase 1 and 2 trials (ALKA-372-001, STARTRK-1 and STARTRK-2). Fast and durable response for treatment was observed across a broad range of solid tumors, including melanoma (94). What is more, entrectinib showed promising antitumor activity in the central nervous system which is particularly important in melanomas regarding their proclivity for central nervous system metastasis (94). This study became the basis for FDA approval in 2019 of entrectinib for adults and adolescents with tumors that test positive for *NTRK* gene changes.

Inhibitors of the heat shock protein Hsp90. Heat shock proteins are a group of proteins whose expression increases when cells are exposed to stress factors. They supervise the processes of creating and protecting the spatial structure of all proteins. Based on their molecular weight, they are divided into five main groups: Low-molecular-weight Hsps, Hsp60s, Hsp70s, Hsp90s and Hsp100s (95). Hsp90s are the most well studied HSPs in cancer as they play important roles in carcinogenesis. They can form protein complexes protecting RAF and AKT, proteins involved in tumor growth (95). When combined with immunotherapy, blocking Hsp90 proteins may be a promising part of the therapeutic strategy due to the enhanced effect of T-cell lymphocytes killing cancer cells, potentiation of therapy targeting checkpoints and enhancement of the functions of cytotoxic lymphocytes when combined with anti-CTLA4 antibodies (96). Hsp inhibitors are intended to inhibit the induction of myeloid-derived suppressor cells. These cells are activated by melanoma cells and are responsible for the suppression of immunity, thus increasing the risk of metastasis (97). Clinical trials confirmed the activity of XL888 in combination with

vemurafenib in patients with a V600 mutation. The possibility of XL888 reducing the resistance developed during treatment with BRAF inhibitors was also indicated (98).

Proteasome inhibitors. Proteasomes are multi-enzymatic complexes involved in the degradation of abnormal proteins. Inhibition of proteasomes causes an accumulation of pathological proteins, activation of caspases and cell death. In the course of cancer, cancer cells become genetically unstable and synthesize abnormal proteins. Blocking the breakdown of such proteins by inhibiting proteasomes causes their accumulation within the cell, resulting in its death through the activation of caspases. Therefore, compounds that inhibit proteasomal activity are currently used in cancer therapy (99).

Bortezomib is used to treat multiple myeloma. However, a phase II trial did not confirm its efficacy in the treatment of disseminated melanoma (100). Despite promising results of preclinical studies, phase I clinical trials did not show any significant activity for the combination of bortezomib and temozolomide (100,101) or for the combination of bortezomib and sorafenib (102).

Other treatment options. Imiquimod, an immune response modulator, can be used in the treatment of inoperable superficial skin lesions by administering it locally to the lesion surface (103). The mechanism of action of imiquimod is to stimulate macrophages and monocytes to produce interferon-α and cytokines (studies have shown its efficacy in reducing cutaneous metastases from the primary) (103). Talimogene laherparepvec is an attenuated herpes simplex virus type-1 (HSV-1) lacking the ICP34.5 and ICP47 genes with an inserted coding sequence for the human granulocyte-macrophage colony-stimulating factor (GM-CSF), which is administered to a neoplastic lesion and captured by HSV-1 receptors on cancer cells and normal cells (104-106). After administration, the virus replicates only in cancer cells. The presence of GM-CSF is intended to additionally stimulate a systemic antitumor immune response and effector T-cell response (104-106). In a phase III study, the drug was compared to subcutaneously administered GM-CSF in patients with stage IIIB, IIIC and IV melanoma who were not eligible for surgery (106). The percentage of permanent response was 16.3% in the drug group and 2.1% in the GM-CSF group. The reduction of untreated metastatic lesions by 50% was observed in 27 out of 79 patients (34.2%) with lesions located outside abdominal organs, and in 8 out of 71 patients (11.3%) with lesions in abdominal organs. The drug was registered in the European Union and in the US for the treatment of adult patients with unresectable melanomas with metastases to local lymph nodes or with distant metastases not involving bones, brain, lungs or other internal organs (stages IIIB/IIIC/IVM1a) (106).

There are also other substances that appear to be promising, for instance: Modulators of Toll-like receptors (TLRs), inhibitors of poly(ADP-ribose) polymerase(PARP), anti-angiogenic agents, monoclonal antibodies against CD40 costimulatory molecules, anti-integrin antibodies or antisense therapy, i.e. therapy involving the use of short fragments of DNA or RNA to silence the expression of disease-causing genes, although the studied compound oblimersen did not show high efficacy (107).

4. Current recommendations for immunotherapy and targeted therapy of melanoma

According to the 2020 guidelines of the National Comprehensive Cancer Network (NCCN) (7), adjuvant treatment outside of a clinical trial is not recommended for patients with stage I or II disease (7).

Stage IIIA (sentinel node positive) is the lowest risk group for which the NCCN Guidelines recommend considering adjuvant treatment. Risk of toxicity is one of the major considerations when deciding whether a patient with stage III disease should receive adjuvant treatment. The recommended drugs for adjuvant therapy in the case of disease with lymph node metastases (stage III) are: Nivolumab, pembrolizumab, dabrafenib or trametinib for patients with a BRAF V600 mutation (7). In stage III patients with satellite or in-transit metastases who have undergone a complete excision to clear margins, it is recommended to supplement systemic therapy with an oncolytic virus, talimogene laherparepvec (T-VEC), administered directly to the lesion (99,101). In addition, in patients with unresectable disease, the recommendations include Bacillus Calmette-Guérin (BCG) vaccines, IFN and IL-2 (7). Imiquimod is recommended for use on the surface of superficial lesions (7,103).

Nivolumab or pembolizumab are recommended for stage IV patients with limited metastases after total resection. The recommended first-line therapy in the case of disseminated or unresectable disease are PD-1 inhibitors (pembrolizumab/nivolumab) or a combination of nivolumab and ipilimumab. The recommended targeted therapy (in the presence of a BRAF V600 mutation) is a combination of dabrafenib+trametinib, vemurafenib+cobimetinib or encorafenib and binimetinib (7). Other recommended regimens include vemurafenib and cobimetinib in combination with atezolizumab in BRAF V600 activating mutation presence (7). Second-line therapy includes pembrolizumab or nivolumab monotherapy or a nivolumab+ipilimumab combination, targeted therapy with dabrafenib+trametinib or vemurafenib+cobimetinib or encorafenib+binimetinib, ipilimumab monotherapy, high doses of IL-2, imatinib in the presence of a mutation activating c-KIT, larotrectinib or entrectinib for *NTRK* gene fusion-positive tumors and binimetinib for NRAS-mutated tumors that have progressed after prior immune checkpoint inhibitor therapy (7).

5. Immunotherapy and targeted therapy of melanoma in Poland

Since novel cancer therapies are still expensive, they are intended only for a certain group of patients. In Poland, immunotherapy and targeted therapy are available within the Drug Reimbursement Programme of the Ministry of Health. These therapeutic programmes define eligibility criteria for treatment, programme exclusion criteria, dosing regimen, method of administration, the list of diagnostic procedures performed at screening for the programme and necessary for treatment monitoring.

Combination of dabrafenib+trametinib or vemurafenib+cobimetinib, or encorafenib+binimetinib can be applied in any line of treatment in patients with advanced melanoma (unresectable

Table I. Some of the current ongoing trials (109).

Drug	Clinical trial	Phase
Cytokines	A study of NKTR-214 combined with nivolumab vs. nivolumab alone in participants with previously untreated inoperable or metastatic melanoma	III
BRAF inhibitors	Encorafenib+binimetinib+pembrolizumab in patients with unresectable or metastatic BRAF V600 mutant melanoma (Germany)	I
	Dabrafenib/trametinib/hydroxochloroquine for advanced pretreated BRAF V600 mutant melanoma (Belgium)	I
	A study to evaluate RAF265, an oral drug administered to subjects with locally advanced or metastatic melanoma (USA)	II
	Induction therapy with vemurafenib and cobimetinib to optimize nivolumab and ipilimumab therapy (Netherlands)	II
MEK inhibitors	BGB324 in combination with pembrolizumab or dabrafenib/trametinib in metastatic melanoma (Norway)	I/II
	Intermittent selumetinib for uveal melanoma (USA)	I
Combination of BRAF and MEK inhibitors	Study of neo-adjuvant use of vemurafenib plus cobimetinib for BRAF mutant melanoma with palpable lymph node metastases (Canada)	II
	Neoadjuvant vemurafenib+cobimetinib+atezolizumab in melanoma: NEO-VC (France)	II
	Neoadjuvant dabrafenib+trametinib for AJCC stage IIIB-C BRAF V600 mutation positive melanoma (Australia)	II
	MCS110 with BRAF/MEK inhibition in patients with melanoma (USA)	II
	Study of dabrafenib+trametinib in the adjuvant treatment of stage III BRAF V600+ melanoma after complete resection to evaluate the impact on pyrexia related outcomes (USA)	III
Farnesyltransferase inhibitors	Tipifarnib in treating patients with metastatic malignant melanoma (USA)	II
KIT inhibitors	Efficacy and safety of nintedanib combined with paclitaxel chemotherapy for patients with BRAF wt metastatic melanoma (Germany)	II
TRK inhibitors	A study to test the safety of the investigational drug selitrectinib in children and adults that may treat cancer (USA)	I/II
Inhibitors of the PI3K/AKT/mTOR pathway	Everolimus in treating patients with stage IV melanoma (USA)	II
	CCI-779 (temsilorimus) in treating patients with metastatic melanoma (USA)	II
Proteasome inhibitors	An expanded cohort trial of bortezomib and sorafenib in advanced malignant melanoma (USA)	I
	Bortezomib, paclitaxel, and carboplatin in treating patients with metastatic melanoma (USA)	I
Anti-CTLA4 antibodies	A national phase IV study with ipilimumab for patients with advanced malignant melanoma (Norway)	IV
	Immunogenicity and biomarker analysis of neoadjuvant ipilimumab for melanoma (USA)	0
	Phase I clinical trial of tremelimumab plus MEDI3617 in patients with unresectable stage III or stage IV melanoma (USA)	I
	Study of the combination of IMCgp100 with durvalumab and/or tremelimumab in cutaneous melanoma (USA)	Ib/II
Antibodies blocking PD-1 and PD-L1 molecules	Efficiacy, safety and tolerability of V937 administered intravenously or intratumorally with pembrolizumab (MK-3475) vs. pembrolizumab alone in participants with advanced/metastatic melanoma (V937-011) (USA)	II
	Cabozatinib and pembrolizumab for advanced metastatic melanoma (USA)	I/II
	Nivolumab in combination with talazoparib in melanoma and mutations in BRCA or BRCA-ness genes (USA)	II
	Ipilimumab and nivolumab with or without hypofractionated radiotherapy in patients with metastatic melanoma (USA)	II
	Immunotherapy with ipilimumab and nivolumab preceded or not by targeted therapy with encorafenib and binimetinib (France, Belgium)	II

Table I. Continued.

Drug	Clinical trial	Phase
Antisense therapy	Dacarbazine with or without oblimersen (G3139) in treating patients with advanced malignant melanoma (USA)	III
Other	Imiquimod and pembrolizumab in treating patients with stage IIIB-IV melanoma	I

BRAF, B-Raf proto-oncogene, serine/threonine kinase; MEK, mitogen-activated protein kinase kinase; KIT, receptor tyrosine kinase (CD117); PD-1, programmed death receptor-1; PD-L1, programmed death-ligand 1; TRK, tropomyosin receptor kinase; PI3K/AKT/mTOR, phosphatidylinositol 3-kinase/serine/threonine kinase/mammalian target of rapamycin; CTLA4, cytotoxic T-lymphocyte antigen 4; BRCA, breast cancer gene.

stage III or stage IV) (108). Another treatment option for this group of patients, independent of the BRAF mutation status, is anti-PD1 antibodies as monotherapy or in combination with an anti-CTLA4 antibody (108). Nivolumab and pembrolizumab in monotherapy or the combination nivolumab+ipilimumab are available as first- or second-line treatment whereas ipilimumab in the second line of treatment (108).

The recommended drug for adjuvant therapy in the case of disease with distant metastases is nivolumab (108). Patients with lymph node metastases can be treated with nivolumab or pemborlizumab. In the presence of a BRAF V600 mutation, patients with stage IIIA, IIIB, IIIC or IIID who have undergone total resection can be treated with a combination of dabrafenib and trametinib (108).

6. Clinical trials in progress

Increased biological understanding and access to innovative therapeutic substances have improved the treatment of melanoma. Many clinical trials on different pathways are in progress. They mainly assess the effectiveness of the combination of drugs that are currently used in melanoma treatment but also aim to compare new drugs to already approved and active agents. As most of these trials are in phase II, we must remain patient for the full results, and hopefully, for another breakthrough in the treatment of melanoma. Table I presents some of currently ongoing trials.

7. Summary

Melanoma is characterized by high mortality, especially at the metastatic stage. The best treatment results are achieved with radical surgical resection of a limited lesion; however, in the case of a disseminated process where surgery is of limited use, systemic treatment is necessary. Treatment of metastatic patients with conventional chemotherapy has not brought the expected results in terms of extending their survival time or improving their comfort. Therefore, there has emerged a need to seek other solutions. Although immunotherapy of melanoma is still in its infancy, many published studies indicate that it is already highly promising. In most cases, the use of immunological treatment or targeted therapy has had a positive impact on survival time and relapse-free survival. However, these periods are still relatively short, and most of the patients diagnosed with advanced melanoma succumb to the disease soon after diagnosis. For this reason, further research and improvement of treatment are needed. The main focus should be placed on antibodies blocking PD-1 and PD-L1 molecules, anti-CTLA4 antibodies and therapy with BRAF and MEK inhibitors, as they provide the greatest benefits and are already included in the treatment guidelines. New drug combinations should also be tested, as combined treatment often has a better effect and a more favorable toxicity profile. However, the main emphasis should still be placed on early detection before the cancer metastasizes, since full recovery in such a situation is almost never achieved. One of the possible reasons may be the generalized immunosuppression usually observed in the advanced stages which is related to the extensive production of cytokines (110).

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Authors' contributions

PSS and LGS were involved in the conceptualization of the study. PS and LGS were involved in project administration. PSS, MC, MJ and LGS were involved in the investigative aspects of the study. PSS, MC, MJ and LGS were involved in data validation. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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