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Current trends in the treatment of malignant melanoma

Minireview

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Currently, skin cancer is one of the most frequent type of cancers. Melanoma is much less common than basal cell and squamous cell skin cancers, but it is far more dangerous. Melanomas represent 3% of all skin cancers but 65% of skin cancer deaths. Detailed knowledge of melanoma at the molecular level allows the development of new treatment alternatives and to design effective new drugs. There are two approaches in therapy of melanoma in the present is based on immunotherapy and targeted therapy or their combination. Immunotherapy includes immune checkpoint blockades whereas targeted therapy is represented by protein kinase inhibitors. Detailed knowledge of protein structure and the understanding of their role in key signalling pathways in melanoma development lead to the designation of new protein kinase inhibitors in targeted therapy. In the future, it is necessary to conduct further clinical trials and collect more data about overall survival, response rates, appropriate timing and sequence of combination therapy to manage the complexity of melanoma treatment.

Key words: melanoma, immunotherapy, targeted therapy, protein kinase inhibitors

The incidence of melanoma is increasing worldwide. Melanomas represent 3% of all skin cancers but 65% of skin cancer deaths [1]. Melanoma is currently the fifth and sixth most common solid malignancy diagnosed in men and women, respectively [2]. The rates of melanoma have been rising for at least 30 years [3]. Although melanoma is no longer considered just 'one disease', pathologists will continue to have important role in to identifying and describing tumor subtypes [4]. More detailed understanding of melanoma allows the development of new specific treatment alternatives, which are targeted at specific receptors or the genes of tumor cells. In 2011, new molecules were discovered and designed on the basis of new knowledge in the molecular biology of melanoma. These new facts have resulted in the existence of two new approaches to therapy – immunotherapy and targeted therapy of melanoma.

Genesis of melanoma

Melanoma is derived from melanocytes – normal pigment cells of the skin. Most commonly, melanoma arises from epidermal skin melanocytes, but primary tumors can be found also lining the choroidal layer of the eye (uveal melanoma) or the mucosal surfaces of the respiratory, genitourinary, and gastrointestinal surfaces [5]. Melanoma is much less common than basal cell and squamous cell skin cancers, but it is far more dangerous. Like basal cell and squamous cell cancers, melanoma is almost always curable in its early stages. However, it is much more likely to spread to other parts of the body than basal or squamous cell cancer if not caught early. Melanocytes that produce melanin are usually uniformly localized at the interface of the dermis and epidermis of the skin. If the melanocytes are found in denser groups, they create different forms of birthmarks – nevus. Human nevi are benign tumors of melanocytes that are frequently associated with oncogenic mutations predominantly in BRAF V600E. However, nevi typically remain in a growth-arrested state for decades and only rarely progress into malignant melanoma. Very important features of nevus include oncogene-induced senescence [6] and oncogene-induced trans-lineage differentiation [7] which prevent benign nevi from malignant transformation.

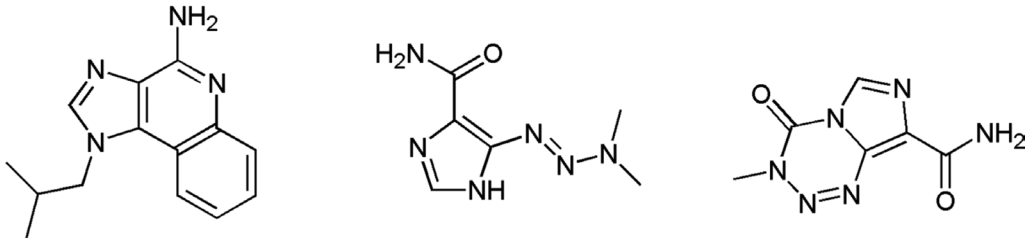

Figure 1. Chemical structures of imiquode, dacarbazine and temozolomide

In recent years, researchers have learned a great deal about how certain changes in DNA can make normal cells to become cancerous. Cancers can be caused by DNA changes that turn on oncogenes or turn off tumor suppressor genes. Changes in several different genes are usually needed for a cell to become cancerous. Damage of DNA may be in the form of inherited genetic mutation, but in most cases it occurs gradually over the life due to the influence of environmental factors, such as UV rays from the sun [8, 9, 10].

There are two melanin pigments synthesised in the melanocytes: eumelanin, a dark brown-black insoluble polymer, and pheomelanin, light red-yellow sulphur containing soluble polymer [11]. Pheomelanin has a weak shielding capacity against ultraviolet radiation compared to eumelanin, and has been shown to amplify ultraviolet-A-induced reactive oxygen species [12]. Mitra et al. suggested that the pheomelanin pigment pathway produces ultraviolet-radiation independent carcinogenic contributions to melanomagenesis by a mechanism of oxidative damage.

Current possibilities for the therapy of melanoma

Similar to other tumors the progressive stage of melanoma is predictive for therapeutic success. Early stage melanomas (thin tumors) result in a 97% 5-year survival rate of the patients, after surgical removal [13].

Surgery and chemotherapy

The treatment of cutaneous melanoma has historically been essentially surgical. Much progress has been made in this area, and the resection margins have been established based on tumor depth. Candidates are also identified for lymphadenectomy, avoiding the morbidity of the procedure in patients who do not require it.

Topical formulations are examined and, where available, skin penetration properties of the various drugs are detailed. New strategies for targeted drug delivery to skin cancers are considered with an emphasis on studies conducted *in vitro* with porcine or human tissue, or in patients.

Imiquimod cream may be used to stimulate the local immune response in early stage melanoma patients (Fig. 1) [14, 15].

The decision to treat melanoma by adjuvant therapy has the opposing arguments: the risk of recurrence, progression and high toxicity, and price of treatment.

The risk of recurrence and death after complete surgical resection of clinically detectable primary cutaneous melanoma ranges from low, intermediate to high risk depending on the stage of disease at diagnosis. This is determined by the depth, ulceration status and mitotic rate of the primary tumor, the presence of regional nodal disease or distant metastasis. For high-risk melanoma, adjuvant therapy is aimed at eradicating melanoma micrometastases that carry an unacceptable risk of mortality from melanoma recurrence. The ultimate goal of adjuvant therapy is to provide a potential cure before progression of melanoma into advanced inoperable stages [16].

A little progress has been made in systemic treatment since the 1970s when the use of dacarbazine was introduced for the treatment of patients with tumor progression or distant metastasis, with disappointing results.

Dacarbazine and ***temozolomide*** (Fig. 1) belong to the group of alkylating agents. These triazene compounds have excellent pharmacokinetic properties and limited toxicity. The active moiety of these drugs is represented by the triazenyl group of three adjacent nitrogen atoms which are responsible for the physico-chemical and antitumor properties of the molecule. Mechanism of action of both compounds is mainly related to the methylation of O^6-guanine, mediated by methyldiazonium ion, a highly reactive derivative. O^6-methylguanine is responsible for incorrect base pairing and damaging of DNA [17]. Dacarbazine is a prodrug structurally related to purines activated by liver microsomes. This chemotherapeutic agent was approved by the FDA (Food and Drug Administration) for the treatment of melanoma, and often regarded as the standard treatment for advanced melanoma. However, therapy with dacarbazine is characterised with low overall response rates (approximately 10 – 15%) and there is no valid evidence of survival benefit [18]. Temozolomide is a monofunctional alkylating agent of the imidotetrazine class. It is stable at the acid pH of the stomach and administered orally with 100% bioavailability [17].

New approaches

For years, the cornerstones of cancer treatment have been surgery, chemotherapy, and radiation therapy. Significant changes occurred in antitumor therapy for disseminated melanoma during the last decade. Detailed knowledge in the molecular biology of melanoma and immune response lead to the two directions – immunotherapy and targeted therapy. Before 2011, two approved drugs were used to treat patients with metastatic melanoma in the USA – dacarbazine and recombinant human interleukin-2 (IL-2) [19]. The treatment landscape for advanced stage melanoma was revolutionized in 2011 with the approval of ipilimumab and vemurafenib, both of which improved overall survival in phase III clinical trials. More recently, the targeted inhibitors dabrafenib and trametinib have demonstrated similar therapeutic profiles [20]. The latest approved (PD-1)-blocking antibody pembrolizumab is indicated for the treatment of patients with unresectable or metastatic melanoma [21].

Immunotherapy

The immune system recruitment may represent a powerful and innovative strategy in cancer therapy. Genetic mutations and alterations in regulatory processes of cancer cells lead to expression of various tumor-related antigens that can be presented to cytotoxic T-lymphocytes by antigen-presenting cells. A major understanding of immune activation, especially T-lymphocyte activation, has identified multiple co-stimulatory and co-inhibitory pathways regulating this process. The two most important targets of immunotherapy are co-inhibitory receptors, such as CTLA-4 (cytotoxic T-lymphocyte-associated antigen 4) and programmed cell death-1 (PD-1) receptor, expressed on the T-lymphocyte surface [21].

A molecule of IL-2 was first approved by the U.S. FDA for immunotherapy of melanoma. It is of limited use due to the serious toxic side effect of this treatment [22]. The first approved checkpoint blocking antibody was ipilimumab.

Ipilimumab (Yervoy®) is a human monoclonal IgG1 antibody that binds the human antigen CTLA-4 located on the surface of T-lymphocytes and blocks its interaction with molecule on the surface of antigen presenting cells. CTLA-4 is a key negative regulator of adaptive immune response and works as a brake on the immune response. Blocking immune response to anticancer leads to a longer and stronger activation of T-lymphocytes and, ideally, to an attack and destruction of the tumor tissue, resulting in long term remission for 15 – 20% of patients [23]. Although its effectiveness is tested with many carcinomas, the best results were achieved just in the treatment of melanoma [24]. Randomized clinical studies show that the treatment with ipilimumab leads to a significant extension of the survival of patients with metastatic melanoma. Side effects of ipilimumab are related to the mechanism of its action. Typical side effects are accompanied by diarrhoea, skin rash, pruritus, enteritis, vitiligo, endocrinopathies and hepatotoxicity. Ipilimumab is approved in the USA for the treatment of patients with advanced melanoma and in Europe for patients with previously treated advanced melanoma.

Tremelimumab, another drug of this group, is human therapeutic monoclonal antibody IgG2, with the same mechanism of action as ipilimumab. This antibody is currently in progress in phase II/III clinical study [25].

Ipilimumab, in combination with high dose IL-2, and tremelimumab, in combination with interferon alfa provide increased overall response rate, progression-free survival, or higher percentage of complete responses. ***Interferon alfa*** is FDA approved in adjuvant treatment for patients with high-risk melanoma and it has significant immunomodulatory effects [26, 27, 28]. Interferon alfa monotherapy has limited utility in the treatment of stage IV melanoma, therefore its antitumor activity has led to profound investigation of its use in combination with other therapies [29].

Cancer immunotherapy can be achieved by inhibition of the PD-1/PD-Ll axes which affect the overall survival in an important fraction of patients. PD-1 is an inhibitory receptor that is upregulated on activated lymphocytes. PD-1 has two known ligands, PD-L1 and PD-L2, which can be expressed on tumor and stromal cells; PD-L1 expression can be induced by cytokines produced by tumor-infiltrating lymphocytes [30].

Pembrolizumab (Keytruda®, Merck & Co) is the first anti-PD-1 immunotherapeutic agent approved by FDA. Keytruda® was granted FDA approval on September 4, 2014 for the treatment of patients with unresectable or metastatic melanoma. This molecule is a potent and highly selective humanized monoclonal antibody of IgG4-kappa isotype, designed to directly block the interaction between PD-1 receptor, expressed on T-cells, and its ligands, PD-L1 and PD-L2, without antibody-dependent cell-mediated or complement-dependent cytotoxicity. In practice, blocking PD-1 activity is believed to prevent inhibition of T-cell immune surveillance of tumors and, in some models, has resulted in decreased tumor growth [21]. The recommended dose of pembrolizumab is 2 mg/kg administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression or unacceptable toxicity. Most common adverse reactions (reported in ≥ 20% of patients) included fatigue, cough, nausea, pruritus, rash, decreased appetite, constipation, arthralgia, and diarrhoea [31].

Another approach, which has already been tested, is to combine anti-PD-1 and anti-CTLA-4 treatment and is represented by ***nivolumab*** (Opdivo®, Bristol-Meyers Squibb). Nivolumab is used alone or in combination with ipilimumab [32, 33]. Combination therapy with anti-CTLA-4 and anti-PD-1 monoclonal antibodies has recently led to remarkable antitumor effects, long-term survival and potential cures [34].

Except these immunological checkpoint blockades the approach of ***adoptive T cell therapy*** seems to be a highly promising in use against cancer including melanoma. The ability of T cells to specifically lyse tumor cells and secrete cytokines to recruit and support immunity against cancer make them an attractive proposition for therapy. Since the first idea in 1989 to genetically redirect T cells, a lot of experiments have been

performed. Recent methods of generating tumor-specific T cells include the genetic modification of patient´s lymphocytes with receptors to endow them with tumor specificity. These T cells are then expanded *in vitro* followed by infusion of the patient in adoptive cell transfer protocols. Genes used to modify T cells include those encoding T-cell receptors and chimeric antigen receptors. Several trials with gene-modified T cells are ongoing and some remarkable responses have been reported. Recently published results of complete response of hematologic malignancies to adoptive T cell therapy provide chance for the use of genetically modified T cell also for treatment of melanoma [35].

Targeted therapy

Selected somatic changes such as BRAF mutations have been described, and then applied to the targeted treatments. BRAF gene is located in chromosomal region 7q34; it consists of 18 exons and transcribed mRNA length was 2478 bp. Targeted therapy is based on the knowledge of the molecular biology of the gene encoding the BRAF kinase, belonging to the RAF kinase family. It is a serine/threonine kinase that takes part in the MAPK (Mitogen Activated Protein Kinase) cascade which modulates cell growth and proliferation. This pathway is activated by binding of the extracellular physiological growth factor to its receptor. Conformational change of the receptor leads to the activation of RAS protein (GTP-binding), which activates RAF protein, which activates other kinases MEK and ERK. This pathway may be activated by mutation of specific proteins, including BRAF [36]. It is reported that 40 – 60% of melanomas have a mutation of the gene leading to the pathological-activated signalling pathways and to uncontrolled growth of malignant transformed cells [37]. The most common gene mutations are V600E or V600K known as an amino acid substitution at position 600 in BRAF, from a valine (V) to a glutamic acid (E) or to a lysine (K), respectively. In the structure of protein kinases there is a DFG motif, which is a highly specific site for interaction with kinase inhibitors. It contains Asp (D), Phe (F) and Gly (G) and exists in a conformational active or inactive state. Just the knowledge in this field has led to the development and screening of new selective inhibitors of BRAF and MEK (Fig. 2) [38]. Targeted therapy is associated with improved clinical benefit, however, the mechanism of resistance often varies and includes activation of alternative signalling pathways [39].

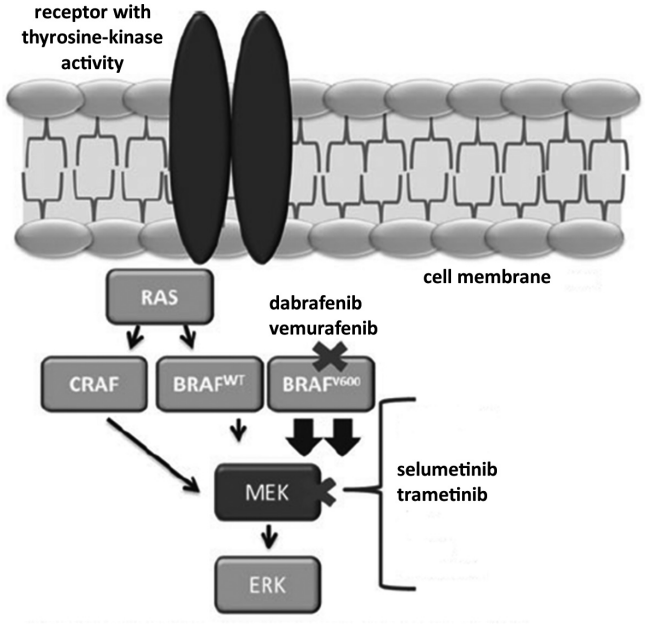

Figure 2. Mechanism of action of kinase inhibitors. This figure shows a schema of signaling pathways triggered by binding of growth factors to tyrosine kinase receptor that triggers RAS, RAF, MEK and ERK pathways leading to cell growth and proliferation. Mutations in BRAF (V600E) can lead to accelerated cell growth and cancer formation of melanoma cells. Inhibition of mutant BRAF by dabrafenib, vemurafenib in the melanoma cells shuts down the signaling pathway causing tumor regression following cell apoptosis, tumor antigen expression and decreased release of cytokines and VEGF. MEK is a member of the MAPK signaling cascade that is activated in melanoma. Inhibition of MEK by selumetinib, trametinib blocks cell proliferation and induces apoptosis (controlled cell death). MAPK, Mitogen Activated Protein Kinase; ERK, Extracellular Signal-Regulated Kinase; VEGF, Vascular Endothelial Growth Factor.

BRAF inhibitors

Vemurafenib (Zelboraf® tablets, Roche) is the first selective inhibitor of BRAF developed by Plexxikon and approved by FDA in 2011 (Fig.3). It leads to a rapid, and sometimes the complete remission of the disease in patients with a mutated BRAF V600E. A clinical study on 675 respondents treated with vemurafenib, 960 mg twice daily, demonstrated survival of 6 months in 84% of patients versus 64% of patients treated with dacarbazine. Despite significant benefit in the treatment, there were new challenges identified – the development of resistance to reactivation of MAPK signalling and growth of keratoacanthomas and squamous cells. The most common adverse events were headache, joint pain, fatigue, skin hyperkeratosis and 6% of the patients experienced a squamous cell carcinoma [40].

Dabrafenib (Tafinlar® capsules), developed by GlaxoSmithKline (Fig. 3), selectively inhibits BRAF ValGlu [41]. It is a thiazole derivative, which binds to the ATP binding site of BRAF kinase. It has a shorter half-life than vemurafenib (5.2 h versus 50 h). In 2009, first clinical studies in Phase I/II began. In Phase III clinical trials, the dosing regimen was 150 mg of dabrafenib twice daily which significantly extended the survival to 5.1 months versus 2.7 months with dacarbazine. Hyperkeratosis, headache and joint pain, fatigue, heartburn have been reported as adverse events [19].

MEK inhibitors

Trametinib (Mekinist® tablets, GlaxoSmithKline) is the first selective allosteric inhibitor of MEK1 and MEK2 (Fig. 3). In May 2013, it was approved by the FDA as a single agent for the treatment of patients with V600E mutated metastatic melanoma [42]. The recommended daily dose of trametinib

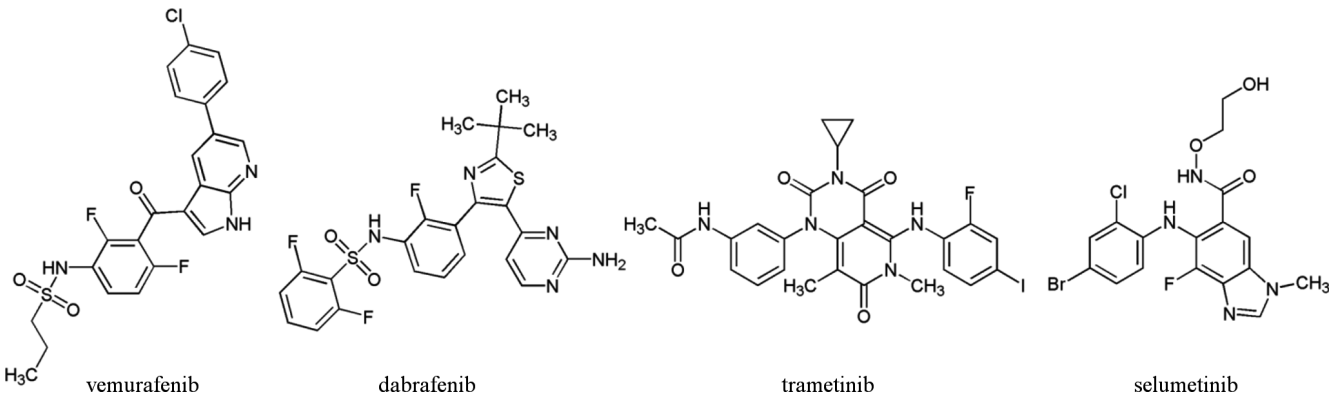

Figure 3. Chemical structures of vemurafenib, dabrafenib, trametinib, selumetinib

is 2 mg orally daily. It has a long half-life, i.e. four days at the previously mentioned dosing. In Phase III clinical study, trametinib was well tolerated by patients who most commonly experienced side effects such as diarrhoea, asthenia, rash, nausea and vomiting [43]. Development of squamous cell carcinoma as a side effect did not occur at all unlike in treatment with BRAF inhibitors [19].

Selumetinib, licensed by Array BioPharma Inc to AstraZeneca in 2003, inhibits the MEK enzyme in the RAS/RAF/MEK/ERK pathway in cancer cells to prevent the tumor from growing (Fig. 3). In April 2015, selumetinib was granted Orphan Drug Designation by the U.S. FDA in recognition of the need for new, safe and effective therapies for the uveal melanoma [44]. Uveal melanoma is a rare disease in which cancer cells form in the tissues of the eye. It is the most common primary intraocular malignancy in adults and comprises 5% of all melanomas [45]. In July 2015, AstraZeneca announced that the Phase III clinical SUMIT study of selumetinib in combination with dacarbazine for the treatment of patients with metastatic uveal melanoma did not meet its primary endpoint of progression-free survival. This combination therapy showed an adverse event profile generally consistent with current knowledge of the safety profiles of dacarbazine and selumetinib [46].

Currently, there are being conducted ongoing studies in the elimination of resistance of the MAPK cascade by concomitant administration of inhibitors of MEK and BRAF [36]. This combination of BRAF and MEK inhibitors may prolong progression-free survival, and consequently increase the overall survival of patients. Therapy reactions or responses in patients may be different; the anti-CTLA-4 immunotherapy may lead to long-term response, but not in all patients, whereas targeted drugs may cause responses in most patients, though almost all of them eventually experience relapses due to preexisting or acquired resistance.

A wide range of mutations are known to prevent effective treatment with chemotherapeutic drugs. Hence, approaches with biopharmaceuticals including proteins, like antibodies or cytokines, are applied [5]. Modern therapeutic approaches in melanoma provide profound and long lasting effects and can even cure some patients. Rational consecutive and combined application of current methods, proper diagnostic and management of related adverse events can prolong life span of patients and meaningfully increase their quality of life [47].

Combination immunotherapy and targeted therapy

Studies about combinations of anti-PD-1/PD-L1 agents with other immunotherapeutic agents are currently conducted in treatment of multiple tumor types. Targeting immune checkpoints such as PD-1, PDL-1 and CTLA-4 has achieved remarkable benefit in multiple cancers by blocking immunoinhibitory signals and enabling patients to produce an effective antitumor response. Inhibitors of CTLA-4, PD-1 or PDL-1 administered as single agents have resulted in durable tumor regression in some patients, and combinations of PD-1 and CTLA-4 inhibitors may even enhance antitumor benefit [48]. The combination of ipilimumab and nivolumab was studied in a phase I trial of 86 patients with pretreated malignant melanoma and demonstrated a 40% objective response rate [49]. In phase II [50] and III studies [51] of this combination used in the treatment of advanced melanoma response rates were quite impressive, but toxicity was notably increased. 83-89% of patients required either topical or oral immunosuppressive therapy for immune-related adverse events (irAE) which led to treatment discontinuation in 36-47% of all patients [50, 51]. However, almost all of the patients (80-100%) treated with immunosuppressive agents had their irAE completely resolved [52].

Recent study by Kim et al. suggests that the addition of MEK inhibitors to targeted and immunotherapy combinations may be associated with increased toxicity; several patients treated by dabrafenib (BRAF inhibitor), trametinib (MEK inhibitor), and ipilimumab (CTLA-4 inhibitor) developed adverse events related to colonic perforation. This condition found in several patients increases the need to further understand the immunomodulatory effects of trametinib [53].

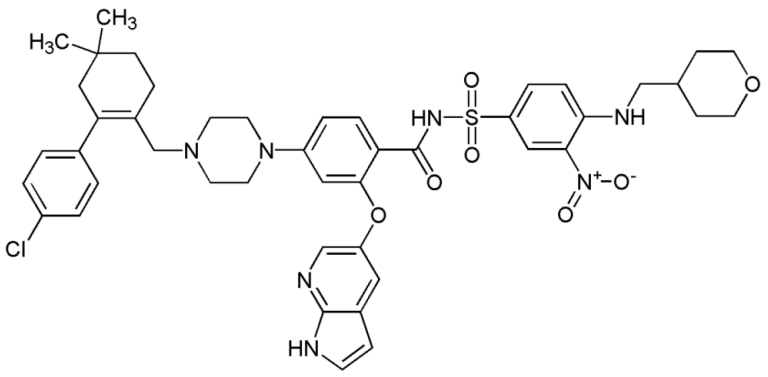

Figure 4. Chemical structure of venetoclax

The optimal timing and sequence of combination therapy (in particular targeted therapy in combination with immunotherapy) is currently in progress and cannot be precisely predicted for all patients with melanoma. Due to the existence of many potential targets in the immune system many critical questions arise, e.g. which therapy combinations should move forward in development and which patients will benefit from these treatments [48].

Future direction in targeted therapy

Despite extensive new approaches in the treatment of advanced stage melanoma, i.e chemotherapy, targeted therapy and immunotherapy, response rate is rarely higher than 20%. Especially in the treatment with BRAF inhibitors the drug resistance is very common [54]. Due to this reason there is an urgent need to invent other alternatives and targeted therapies. Preclinical studies looking at least this main drug association strategies seems to be very promising: targeting of either MEK or phosphatidylinositol-3 kinase (PI3K)/mammalian target of rapamycin (mTOR); strategies aimed at blocking anti-apoptotic proteins belonging to B-cell lymphoma (BCL-2) or inhibitors of apoptosis (IAP) families associated with MEK/BRAF/p38 inhibition; co-inhibition of other molecules important for survival (proteasome, histone deacetylase and signal transducers and activators of transcription) [55]. ***PI3K-AKT-mammalian target*** of ramapycin signaling pathway is important for melanoma initiation and progression so the precilinical investigation of a novel and highly potent PI3K-mTOR dual inhibitor ***VS-5584*** was realized. VS-5584 induced caspase-dependent apoptotic death in melanoma cells, and its cytotoxicity was alleviated by the caspase inhibitors [56]. Whereas the main aim of inhibiting MAPK signaling pathway is to prevent cancer cell proliferation, apoptosis is controlled by the availability of anti-apoptotic ***BCL-2 proteins*** (e.g. BCL-2), which reside at the outer mitochondrial membrane. BCL-2 supports neoplastic growth by blocking cell death and this target may be future direction in the treatment of various type of cancers [57]. Development of small molecule inhibitors specific for antiapoptotic BCL-2 proteins is a novel approach not only for therapy of chronic lymphocytic leukemia [58] but is very promising in therapy of advanced melanoma [59]. This new targeted approach could by more succesful when the combination with retinoid derivative is used [60]. ***Venetoclax (ABT-199)*** (Fig. 4) is the first orally bioavailable selective inhibitor of BCL-2 protein often over-expressed in chronic lymphotic leukemia (CLL) and other types of B-cell related cancers developed by AbbVie in partnership with Roche. It is currently being evaluated in Phase II and Phase III studies for CLL and in Phase I and II studies for several other blood cancers and can be one of the next molecules used in the treatment of melanoma in the near future [61].

In the field of other genetic abnormalities such as ***CDKN2A*** also known as **cyclin-dependent kinase inhibitor 2A, EGF** (epidermal growth factor, which plays a role in skin cell growth), ***Fas* gene,** tumor suppressor gene ***PTEN*** (phosphatase and tensin homologue), there is a challenge in the research of new therapeutic targets and development of new anti-melanoma drugs in the future which can eventually lead to therapeutic benefit. Recent study by Hodis et al. describes six novel melanoma genes (PPP6C, RAC1, SNX31, TACC1, STK19, and ARID2), three of which RAC1, PPP6C, and STK19 harbored recurrent and potentially targetable mutations [62]. The prevalence of BRAFV600 and KIT mutations were significantly associated with melanoma subtypes and BRAFV600 and TP53 mutations were significantly associated with cutaneous primary tumor location. These results enrich understanding of the patterns and clinical associations of oncogenic mutations in melanoma which could be the goal of future direction of melanoma therapy [63].

Conclusion

The development of new drugs in the treatment of melanoma has never been as intense as at present. Single-agent chemotherapy is considered to have rather palliative effect on patients with melanoma; it is usually well tolerated but is associated with lower response rate. Detailed knowledge of protein structure and the understanding of their role in key signalling

pathways in melanoma development lead to the designation of new targets for treatment of melanoma. Although the concept of a combination of immunotherapeutic and targeted agents appears to be crucial in the treatment of melanoma, the synergy between these two approaches in melanoma treatment remains controversial due to the potential increased toxicity. In the future, it is necessary to conduct further clinical trials and collect more data about overall survival, response rates, appropriate timing and sequence of combination therapy to manage the complexity of melanoma treatment.

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