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Current Therapies and New Pharmacologic Targets for Metastatic Melanoma

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1. Introduction

Malignant melanoma is a tumor that originates from the melanocytes and manifests mainly on the skin. Melanomas are usually strongly pigmented, however amelanotic forms have been observed. Relative to the tumor mass, melanomas have an early tendency to metastasize; the prognosis is therefore poor. Malignant melanoma is responsible for about 90% of skin cancer related mortality [1]. Several risk factors for the development of melanoma have been identified. Ethnic origin is particularly important with a disease that mostly affects people of European origin. Epidemiological studies have shown that exposure to the sun, especially in childhood, is a major environmental cause of melanoma, and that up to 65% of the cases of melanoma are frequently related to sunburn [2,3].

While it is easy for cancer prevention, the incidence of melanoma has increased dramatically over the last four decades and melanoma is now one of the most common forms of cancer, with significant morbidity and high mortality due to its propensity to metastasize. It has a high propensity for haematogenous and lymphatic dissemination to regional and distant sites and is poorly responsive to most systemic therapies. The 5-year survival rate for metastatic melanoma is dismal, ranging from 5% to 10% with a median survival of less than eight months with treatment [4,5].

The therapies used in advanced cases, such as chemotherapy, radiotherapy, biochemotherapy and vaccine, seem unable to provide a cure or improved survival of becoming a melanoma tumors most refractory to treatment [6,7]. Although surgery and radiation therapy are very important in the treatment of metastatic disease, systemic treatment remains the basis of

therapy for the majority of patients, including chemotherapy, immunotherapy, or a combination of approaches such as biochemotherapy [8]. Many compounds have been investigated for antitumor activity in melanoma, but the current treatment options for patients with metastatic disease are limited and non-curative in the majority of cases.

Chemotherapy with cytotoxic agents has been used for the treatment of metastatic melanoma for over three decades. Among the antitumoral agents, efficacy is modest in metastatic melanoma including alkylating agents (dacarbazine, temozolomide, nitrosoureas), analogues of platinum (cisplatin, carboplatin) and microtubular toxins (vincas and taxanes), which have been used alone or in combination [8]. Specifically in relation to cytotoxic chemotherapy, dacarbazine used as a chemotherapeutic agent still represents the single most common option [8]. It was demonstrated that combinations of cytotoxic agents can produce low response rates, although higher than monotherapy with dacarbazine, which are associated with increased toxicity and do not extend significantly the survival of patients [8]. Although of limited therapeutic effect, dacarbazine cannot be dismissed as standard therapy for disseminated melanoma, as in evidence-based analysis no other regimen had greater benefits for the patients' survival [9]. By 2011, only two drugs for metastatic melanoma therapy had been approved by the Food and Drug Administration (FDA), which were dacarbazine and a high dose of interleukin 2 (IL-2). In 2011, the FDA approval of vemurafenib (chemotherapy agent) and ipilimumab (monoclonal antibody) for clinical studies raised optimism for the treatment of metastatic melanoma, since the therapies used until then had had serious limitations, and thus the use of novel strategies for melanoma treatment seemed promising. However, the use of these drugs is limited [10]. The strategies to increase the responsiveness of new therapies are the association with other drugs currently in clinical use, such as dacarbazine, although these therapies are under investigation in vitro and in vivo models.

Other new chemotherapies, including drugs that target biological receptors, are currently in development. Among these are bevacizumab, an endothelial growth factor antibody, and sorafenib, a cellular pathway inhibitor [11]. These agents have shown some efficacy in early clinical trials. The lenalidomide is a thalidomide derivative designed to be more effective and less toxic [12]. In a phase I trial, it was found to be well tolerated by patients with metastatic melanoma and to produce immune activation. Another agent of interest that has shown promising results are the anti Bcl-2 antisense (oblimersen), tested in metastatic melanoma. A combination of Bcl-2 antisense and dacarbazine showed better response than dacarbazine alone, although no significant improvement in overall survival was observed [13,14].

Several clinical trials are being carried out to investigate the antitumor efficacy of new agents as well as diverse immunosuppressive therapeutic strategies, including the use of dendritic cells, high-doses of interferon- α (IFN- α) and/or IL-2 and anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) antibody, alone or in combination with current therapies for treating locally advanced, metastatic and recurrent melanomas [15].

Significant advancements made in the last few years have provided important information on the molecular signalling pathways and gene products, which are frequently deregulated in melanoma stem/progenitor cells and their progenies during melanoma formation and progression to locally advanced and metastatic disease states. Consequently, the combination

of different molecular biomarkers or cytotoxic agents targeting distinct gene products altered during melanoma development may constitute more promising therapeutic strategies. Several other signal transduction pathways have been found to be constitutively active or mutated in other subsets of melanoma tumors that are potentially targetable with new agents. Among these, NF- κ B is another pathway that melanoma tumors use to achieve survival, proliferation, resistance to apoptosis and metastasis. In fact, it has been demonstrated that up regulation of the NF- κ B levels is involved in both the progression of melanoma and an increase of its metastatic potential. Therefore, inhibition of NF- κ B activation seems to be a promising target for anti-cancer therapies [16,17].

Recent studies have shown that proteasome inhibitors such as bortezomib represent a new class of anticancer agents by degradation inhibition of cell cycle regulatory proteins, such as cyclin-dependent kinase inhibitors and I- κ B protein. The efficacy of bortezomib was recently explored in another 2-stage phase 2 clinical trials in combination with other chemotherapeutic agents, including paclitaxel and carboplatin, in patients with advanced solid tumors. The results from these studies evidenced narrow clinical benefit and significant toxicity, hindrances that limit the use of bortezomib [18].

Another compound known to exert numerous pharmacological properties - mostly antioxidant, anti-inflammatory and antiproliferative - is curcumin, a polyphenol present in *Curcuma longa*, a species belonging to the ginger family (Zingiberaceae). With regard to antiproliferative activity, it showed pro-apoptotic activity in a variety of tumors in vitro. To achieve these results, curcumin was used in experiments based on melanoma either in vitro or in vivo models. The importance of curcumin also lies in the fact that this drug seems to reduce the metastatic potential of melanoma, which is the main cause of death [19].

Understanding and overcoming resistance pathways by combining current and future agents, identifying biomarkers to improve therapy and discover new therapeutic targets are promising advances in the treatment of melanoma. In light of this, the purpose of this chapter is to summarize the recent advances in the treatment of metastatic melanoma and to describe the current limitations, as well as to comment on promising future strategies to overcome the limitations.

2. Single agent chemotherapy

Melanoma is considered a chemotherapy-resistant tumor, but in fact several chemotherapeutic agents show single-agent activity at the level of 10% to 15%, similar to the efficacy of the chemotherapeutic armamentarium used against other tumor types. Several combination chemotherapy regimens have been tested, but no survival benefit has been demonstrated. Few of these trials have been compared with standard dacarbazine (DTIC) in an adequately powered randomized trial, and even the most extensive of these trials only aimed to detect unrealistically large improvements in overall survival [20]. For the systemic monotherapy of advanced melanoma, several substances are available whose clinical efficacy is comparable. Palliative monochemotherapy can shrink tumors and thus achieve a reduction in tumor-

related symptoms. The more active agents are the alkylants (dacarbazine, temozolomide, fotemustine, carmustine, semustine), platinum drugs (cisplatin and carboplatin), vinca alkaloids (vindezine and vinblastine), taxanes (docetaxel and paclitaxel), and tamoxifen [7,21]. Table 1 shows the drugs and dosages for monotherapy to melanoma.

Drugs	Dosage	Overall response
Dacarbazine	250 mg/m ² /day for 5 days every 3-4 weeks	5.3 – 25%
Temozolomide	150-200 mg/m ² /day for 5 days every 4 weeks	13.5-21%
Carmustine	75-110 mg/m ²	13-18%
Semustine	130 mg/m ²	16%
Fotemustine	100 mg/m ² /day every 3 weeks	7.4-25%
Cisplatin	60-150 mg/m ²	15%
Carboplatin	400 mg/m ² every 4 weeks	19%
Vindezine	3 mg/m ² every 14 days	12-26%
Vinblastine	6-8 mg/m ² every 1 week	13%
Docetaxel	100 mg/m ² every 21 days	14%
Paclitaxel	125-275 mg/m ²	15%
Tamoxifen	20 mg/day	4-13%
Interferon α	9m-18m IU/m ² 3x/week continuous administration	13-25%
Interleukin-2	600 000 IU/Kg as 15 minute short infusion i.v. every 8 hours on days 1-5 16-21.6% (maximum 14 individual doses) repeat cycle day 14	

Adapted by Garbe et al. (2008) [21] and Mouawad et al. (2010) [7].

Table 1. Single drugs, dosage and their activity in melanoma.

2.1. Alkylants agents

2.1.1. Triazines

The alkylating agent dacarbazine (DTIC, dimethyltriazeno imidazole carboxamide) was first introduced some 30 years ago, and it is considered to be the reference of single agent for the management of advanced melanoma. This compound was initially designed as an antimetabolite because it is an analogue of 5-aminoimidazole-4-carboxamide, an intermediate in purine biosynthesis. However, it presented cytotoxicity that is related to the generation of methyl diazonium, which methylates DNA during its metabolism [22]. Methyl diazonium has a half-life of about 0.4 s in aqueous solution, sufficient time to allow it to reach its target. In 1975 this carboxamide was approved by the Food and Drug Administration (FDA), and has since been considered the standard of metastatic melanoma care. Single-agent DTIC has remained the standard of care for 3 decades, with response

rates of 8% to 20% and mean duration of response of approximately four to six months. Several studies of dacarbazine in the management of metastatic melanoma were published in the early 1970s [23,24]. The first of these trials demonstrated a 19% response rate in 110 evaluable patients who received dacarbazine in 3-weekly cycles, most frequently at a dose of 250 mg/m² daily for five days [25]. A second study showed an overall response rate of 28% in 115 evaluable patients receiving dacarbazine 2.5 or 4.5 mg/kg daily for ten days of a thirty-day cycle, and another showed response rates approaching 20% with dacarbazine 150 mg/m² daily for five days of a thirty-day cycle in 112 evaluable patients [24]. The typical dacarbazine dose is 850 to 1,000 mg/m² every three weeks [26].

Among the randomized trials in which dacarbazine was used as a comparator arm, the patients treated with dacarbazine with an overall response rate of 13.4% and median survivals ranging from about six months to eleven months. Given the low response rate, it is unrealistic to expect dacarbazine to have an effect on median survival [27].

Studies suggest that less than 5% of patients achieve a complete response to therapies with dacarbazine alone or in combination, and less than 2% of patients survive five years more. The majority of previous reports suggest that long-term survival is restricted to patients with favourable clinical features, such as nodal, cutaneous or pulmonary metastasis [28].

Although dacarbazine presents a relatively small therapeutic response, it is still considered the standard drug for the treatment of melanoma, whereas the drug combination and the use of other drugs do not show effect as monotherapy.

Another problem with associated dacarbazine is the high toxicity. The major toxic effects associated with dacarbazine are the development of neutropenia, thrombocytopenia, alopecia, nausea or vomiting, fatigue and liver damage [29].

Besides its toxicity, dacarbazine has several drawbacks because of its hydrophilicity, which leads to slow and incomplete oral absorption, and therefore the alternative is intravenous administration. Another disadvantage is its high photosensitivity, with a very short half-life (about 30 min), decomposing to 2-azahypoxanthine via an intermediate diazonium species. For this reason, intravenous infusion bags of dacarbazine must be protected from light. These problems have stimulated the synthesis of dacarbazine analogues, the most important of which is temozolomide [20].

Temozolomide is an oral alkylated compound similar to dacarbazine (the most active single agent in primary melanoma), which has 100% of oral bioavailability and considerable penetration in Central Nervous System tissue, mainly due to its lipophilic character [30]. Moreover, for its acid-stability the molecule remains in cerebro-spinal fluid, where it reaches 30–40% of plasmatic concentration [31]. For this reason, differently from dacarbazine, temozolomide is employed in the treatment of primitive [32] and metastatic brain tumors [31]. Although the FDA has not approved it for melanoma, temozolomide is widely used and has response rates similar to dacarbazine in melanoma [26,33].

Despite the absence of approval, temozolomide is also used “off-label” in patients with malignant melanoma after standard treatment. Temozolomide has been proven to have an

equal efficacy (with a 10–20% response rate) to that of dacarbazine, the most commonly used chemotherapy agent in metastatic melanoma, in a randomized phase III trial [34,35]. However, TMZ administered in monotherapy did not show significant improvement in the overall response rate or median survival time. Nonetheless, the works of Biswas et al. (2006) [36], Chang et al. (2007) [37], Platta et al. (2007) [38] and Mehta et al. (2010) [39] show the efficacy and modest side effects of single-agent TMZ in patients with recurrent or progressive brain metastases.

In a randomized trial comparing temozolomide given for five days every month with dacarbazine given once every 3 weeks, there was no difference in response rate or survival [26]. Despite this, temozolomide offers two potential advantages over dacarbazine. Temozolomide readily crosses the intact blood-brain barrier, which provides enhanced activity against brain metastases. Unfortunately, the objective response rate of melanoma brain metastases to temozolomide is low [37], although there is some indication that treatment with temozolomide is associated with a lower incidence of disease progression in the brain [38,39]. Another potential advantage of temozolomide is that, as an oral agent, continuous dosing is feasible. An extended-dosing schedule of 75 mg/m²/day for 42 days followed by 14 days off has been used in several clinical trials. This schedule provides six weeks of continuous drug exposure and delivers 50% more drug over two months compared with the standard schedule of five days every month. However, a phase II trial using extended-dosing temozolomide showed only a 12.5% response rate [40], which is not different from what would be expected with standard-dosing of temozolomide or of dacarbazine.

Patients that received oral temozolomide at a starting dosage of 200 mg/m²/d for five days every 28 days showed median survival time of about eight months, while patients treated with dacarbazine of about seven months. These results showed that treatment with temozolomide presented significantly longer survival, nevertheless no major difference in drug safety was observed. Temozolomide was well tolerated and produced a noncumulative, transient myelosuppression late in the 28-day cycle. The most common nonhematologic toxicities observed were mild to moderate nausea and vomiting, which were easily managed. Temozolomide therapy improved health-related life quality [26].

The most common hematologic side-effect of temozolomide is myelosuppression (similar to that produced by dacarbazine), particularly neutropenia and thrombocytopenia (dose-limiting toxicity). In contrast, nausea and vomiting are limited (10–15%), whereas the same side effects are remarkably severe and very frequent (i.e. of the order of 90%) in patients treated with dacarbazine. Both drugs can induce headache, fatigue, liver toxicity and constipation [34].

Nevertheless, the development of resistance against the treatment is very common. There are likely multiple mechanisms of chemotherapy resistance specifically for dacarbazine or temozolomide, such as DNA repair of the adduct formation (O⁶-guanine) with the O⁶-methylguanine-DNA methyltransferase [26,41]. Other mechanisms of resistance include Bcl-2 overexpression, silencing of *apoptotic protease activating factor-1* gene (a key mediator of apoptosis), and activation of proliferative and antiapoptotic signalling pathways, including the mitogen-activated protein kinase and phosphoinositide 3-kinase/Akt pathways [34,35,42–44]. In addition, NF- κ B activation of its down-stream target genes (angiogen-

esis, adhesion, antiapoptosis, and chemokine growth factors) likely plays a central role in the proliferation and chemotherapy resistance observed in melanoma [45-47]. It is well established that chemotherapy itself can induce NF- κ B activation in cancer cells [46,47]. Any number of these or other biological processes are likely important to chemotherapy resistance in melanoma.

In an attempt to reduce resistance to chemotherapy in patients with melanoma, new therapies are introduced including the combination of temozolomide and dacarbazine with other chemotherapeutic drugs that have similar mechanism of action or act in different ways. Alternatives used in polychemotherapy in treating melanoma or as monotherapy are the nitrosoureas such as fotemustine.

2.1.2. Nitrosoureas

The nitrosoureas (carmustine, lomustine, semustine and fotemustine) all have single agent activity in melanoma because they cross the blood-brain barrier, thus enabling its use in the treatment of metastatic melanoma. However, at conventional doses, little or no activity was observed against melanoma brain metastases, and overall response rates were low, with only a few complete remissions and short response durations [7,48]. Fotemustine is probably the most active nitrosourea against metastatic melanoma, and especially against cerebral metastasis; its efficacy has been widely demonstrated through several phase II studies.

In patients without brain metastasis treated with fotemustine, the median time for the development of brain metastasis was longer as compared to patients treated with dacarbazine (22.7 months vs. 7.2 months) [49]. It has been widely tested in Europe and has shown overall response of 20-25%, including 5-8% of complete response rates, and it was the first drug to show significant efficacy in brain metastases [50,51].

A multicenter trial of monotherapy was undertaken in 153 evaluable French patients, in which a response rate of 24.2% was obtained. In phase II studies fotemustine was administered intravenously as a rapid infusion, at a dose of 100 mg/m² on days 1, 8, 15, then after four to five weeks every three weeks for maintenance. These studies showed response rates between 16% and 47% in non-visceral metastasis, and long-term survival without metastasis was reported [50,52,53].

In a phase III clinical trial of fotemustine (100 mg/m² weekly for three weeks) versus dacarbazine (250 mg/m²/ day for five days every four weeks), the response rate for fotemustine was 15.2% versus 6.8% for dacarbazine [49].

Previous preclinical studies have indicated that fotemustine inhibits enzymes involved in the ribonucleotide reduction pathway (i.e. DNA synthesis), whereby responding patients appeared to favour the thioredoxin reductase/thioredoxin electron transfer to ribonucleotide reductase, whereas non-responders expressed the alternate glutathione reductase/glutaredoxin mechanism. The 47% response rate obtained in these studies vs. the 24% reported previously for fotemustine may reflect variations in enzymes in the ribonucleotide reduction pathway in different patients. However, the efficacy of fotemustine against advanced melanoma warrants

more extensive trials of this drug, especially because the quality of life of the patients during and after chemotherapy was not severely affected [54].

Fotemustine was well tolerated by the patients, with the only mild side effects being thrombocytopenia, leukocytopenia in 40% patients, and easily controlled nausea/vomiting [55].

2.2. Platinum derivatives

Cisplatin and carboplatin have shown modest activity as single agent therapy in patients with metastatic melanoma. Cisplatin and carboplatin showed good activity against human melanoma cell line, but during in vivo study it did not improve the response comparing with other drugs used in monotherapy of melanoma treatment. It has been shown that cisplatin induced a 15% response rate with a short median duration of three months [8]. Evidence that the activity of cisplatin may be dose-dependent has come from single institution studies. Cisplatin is also used as a palliative in selected patients with malignant melanoma and central nervous system metastases. It was shown that cisplatin can be administered alone via intracarotid infusion in doses varying from 40 to 75mg/m², but the response is not satisfactory, as only about 13% of the patients presented stabilization of the disease [56]. With regard to carboplatin, in a study on 26 chemotherapy naive metastatic melanoma patients, a response rate of 19% with five partial responses has been reported, and thrombocytopenia was the dose-limiting toxicity [57]. Oxaliplatin is a platinum analogue, which cytotoxic activity observed in an in vitro assay appears to be significantly superior to that of carboplatin. Its activity becomes comparatively closer to that of cisplatin as exposure time increases. Indeed, at a 24 h exposure oxaliplatin appears to be significantly more active than cisplatin [58]. Preclinical data have confirmed its non-overlapping spectrum of activity with cisplatin, including acquired and intrinsic platinum resistant cell lines [59]. In a small phase II trial by Evans et al. (1987) [60], no objective response was observed from 10 patients who had previously failed to respond to chemotherapy.

2.3. Vinca alkaloids

The vinca alkaloids, especially vindesine and vinblastine, are usually used in melanoma treatment. A phase II study by Mohammed et al. (2000) [61] with vinorelbine 30mg/m²/week by intravenous bolus with 21 patients no complete or partial response was observed. The estimated median progression-free survival was two months, and the estimated median overall survival was six months. Patients received vinorelbine at a dose of 30 mg/m² on days one and eight of a 21-day cycle, on an outpatient basis. Thirteen patients were included in the study, and received 64 cycles. All patients were assessable for response, toxicity and survival. The median progression-free survival was 3.3 months and the estimated median overall survival was 8.1 months. Vinorelbine as a single agent on days one and eight of a 21-day cycle has a favourable toxicity profile, but seems to have no relevant clinical activity in patients with metastatic melanoma [62] Vindesine is frequently included in combination regimens for the treatment of malignant melanoma and head and neck cancer, although its single-agent activity in these diseases is modest (average of 14 and 12% response rates, respectively) [63]. Similarly, another study that investigated the usefulness of vindesine monotherapy as salvage therapy in stage IV melanoma patients who had previously failed to respond to chemotherapies also

failed; the treatment with vindesine 3mg/m² given to the patients every two weeks in a median of three treatments was stopped due to the disease progression. The median survival after starting vindesine therapy was four months. In this study the authors concluded that vindesine monotherapy is ineffective in stage IV melanoma patients [64]. A phase II study by Retsas et al. (1979) [68] showed a 30% rate response in 25 patients' treatment with vindesine, but with pronounced side effects. Apart from a low rate response the vinorelbine showed significant toxicity, including febrile neutropenia, granulocytopenia, leukopenia, asthenia, nausea, neuropathy, myalgia, dyspnea and fatigue [61].

2.4. Taxol

Preclinical studies indicate that taxanes disturb the cytoskeleton architecture and stabilize microtubules, causing mitotic arrest [65]. Taxol is a major new antitumor agent with significant activity against a number of human cancers. Preclinical investigation demonstrated significant activity against B16 melanoma and against cells derived from melanoma in a human stem cell assay [66]. Studies in phases II and III have shown that taxol has activity against melanoma, but the responses were partial with 16.2% rate response and median duration of approximately five months [67]. Another study with paclitaxel administered at a dose of 100mg/m² intravenously on day one each week for six weeks, the median survival was 209 days [68]. In a phase I trial with paclitaxel administered at 200 to 275 mg/m² over 24 hours every three weeks, there were four partial responses observed in the 12 patients enrolled [69]. A phase II trial with paclitaxel administered at 250 mg/m² over 24 hours every three weeks in 25 patients resulted in three partial responses (12%); an additional four patients had durable objective regression although failing to qualify for partial response [74]. An additional 28 evaluated patients were studied in a second phase II study with paclitaxel administered at 250 mg/m² over 24 hours. Four patients (14%) had objective responses with three complete responses [70]. In another study phase II clinical trial, patients received paclitaxel at 80 mg/m² over one hour, weekly for three weeks, followed by a one-week rest period; the disease status was assessed every other cycle and the treatment was continued until the patients experienced either disease progression or unacceptable toxicity. All patients were considered to be evaluable for toxicity and all patients were included for response assessment in an intention-to-treat analysis. Patients received paclitaxel for a median of two cycles. None of the 27 patients showed a response to treatment; eight patients had stable disease. The median progression-free survival was 1.8 months and the median survival was 7.6 months [76]. A phase II trial achieved with paclitaxel administered at 90 mg/m² on days one, five, and nine every three weeks demonstrated a 15.6% response in 5/32 patients [71].

Docetaxel showed an average response rate of 11.4% in three phase II clinical trials. In an assay performed by the Enzig group, they administered 100 mg/m² docetaxel every three weeks to chemotherapy naive patients. Two out of 35 (6%) of the patients responded with one complete response. Both these responses lasted longer than two years [65]. In a phase II clinical trial, 38 patients were also treated with 100 mg/m² docetaxel every three weeks and evaluated after two cycles; five partial responses were noted in the 30 evaluable patients (17%) [72].

The most common toxicity of taxois is haematological, mainly related with neutropenia. Additional toxicities included hypersensitivity reaction and diarrhoea, peripheral neuropathy, fatigue, fluid retention and oral mucositis [72,73].

2.5. Tamoxifen

In 1976, Fischer and collaborators reported the presence of estrogen receptors on human melanoma cells derived from metastatic tumors [74]. Following a preliminary report, studies were performed with tamoxifen in the treatment of melanoma, either as a single agent or more commonly in combination with other chemotherapeutic agents. In a meta-analysis study it was demonstrated that tamoxifen does not improve the overall response rate, complete response rate or survival rate when administered as chemotherapy regimens. The rate responses ranged from zero to 10% [75]. However, for women 50 years of age or older treated with tamoxifen alone, a response rate of 25% was observed. Despite these promising results, a subsequent phase II trial published in 1992 showed a response rate of only 4.9% in postmenopausal women treated with tamoxifen 40 mg daily (14). In phase II studies the patients treated with tamoxifen 100 mg/m² did not show objective response [76,77]. Another study by Aamdal et al. (1994) [78] reported that the enzyme tyrosinase could mimic estrogen receptors binding, leading to the possibility that this or other interfering substances could account for apparent estrogen receptors activity and the failure to correlate this activity with response to tamoxifen. A remaining controversial question is the inclusion of the antiestrogen in different active chemotherapy regimens, because clinical investigations on the role of tamoxifen in single agent of melanoma have produced inconclusive results.

2.6. Immunotherapy

The relationship between melanoma and the immune system has been recognized, spontaneous tumor regression in patients with metastatic melanoma have suggested that immunotherapy might have a greater impact on the outcome of metastatic melanoma than in other cancers [7]. Immune based treatment using biologic response modifiers, especially interleukin-2 and interferon- α , which have important roles in both adjuvant therapy and in the treatment of metastatic melanoma, may became an antimelanoma strategy [7,79]. However, the landscape for late-stage treatment options has changed recently, with FDA approval in March 2011 for a new immunotherapy drug ipilimumab for treatment of metastatic melanoma [80]. The Ipilimumab, a humanized monoclonal antibody against CTLA-4, is actually a unique way of enhancing patients' immune response against tumors [81].

2.6.1. Interferon alpha (IFN- α)

Interferons represent a family of glycoproteins with a broad spectrum of effects including antiviral, immunomodulatory, antiproliferative, prodifferentiating and antiangiogenic [82,83].

Interferon- α (INF- α) has shown antitumor activity in metastatic malignant melanoma, both as single-agent therapy and in combination with chemotherapeutic agents. As a single agent, IFN- α yields an objective response rate of approximately 15% of responses with less than 5%

of complete response rates and median response duration of between six and nine months with a maximum of 12 months for the best studies [84,85]. Patients that received IFN- α intravenously for one month and 10 MIU/m² subcutaneously three times per week for 48 weeks showed a significantly increased overall survival rate, with a median follow-up of 6.9 years (Kirkwood et al., 1996)

In a controlled trial of two lower doses of IFN- α conducted in patients in observation or in other regimen of treatment, with an intermediate dose of IFN- α (four weeks with 10 MIU/m² administered five times per week, followed by 10 MIU/m² three times per week for one year or five MIU/m² three times per week for two years) for 13 or 25 months, intermediate dose IFN- α did not significantly improve distant metastasis free interval or overall survival outcomes [28]. Low-dose IFN- α also failed to improve survival outcomes versus observation alone when patients were treated with three MIU/m² two times weekly for six months or three MIU/m² three times weekly for two or three years [86-88]. Clinical data were recorded by IFN- α dose: high (20 MIU/m²), intermediate (5–10 MIU/m²), low (3 MIU/m²), and very low (1 MIU/m²) doses. Groups were also stratified by duration of treatment (6 months, 12–18 months or 24 months). Although there was a statistically significant overall survival benefit for treatment of patients with IFN- α , this assimilation did not find evidence of a clear difference in overall survival with different dose levels or duration of the treatments [89]. The mechanism of the therapeutic effects of IFN- α is not completely known. Additional data from Håkansson et al. (1998) [95] and Yurkovetsky et al. (2007) [96] revealed significant decreases of serum levels of immunosuppressive and tumor angiogenic/growth stimulatory factors and increased levels of antiangiogenic. This study also demonstrated a profile of pro-inflammatory cytokines that may help to predict response to therapy. A fundamental question has been raised regarding whether the benefit of IFNs such as immunologic, antiangiogenic, or other antitumor effects would persist long-term or if it would require prolonged, and perhaps indefinite, exposure to IFN- α [90]. When using IFN- α in the long term, the tolerability needs to be weighed up. IFN- α induces acute flu like symptoms and widespread haematological and nonhaematological organ toxicity, dose-dependent fatigue/anorexia, and neuropsychiatric side effects that may endanger compliance over the course of several years' therapy [21].

2.6.2. Interleukin-2 (IL-2)

IL-2, a natural product secreted by CD⁴⁺ T lymphocytes, was described as a T cell growth factor, which plays a central role in immune regulation. However, IL-2 can also modulate immunological effects by stimulating HLA-restricted or non-restricted cytotoxic cell, activate natural killer cells, B lymphocytes, macrophages and induce lymphokine-activated killer cells in vitro as well as the production of other cytokines [7]. The FDA initially approved IL-2 for the treatment of patients with metastatic melanoma in 1998. High-doses of IL-2 (600.000-720.000 IU/Kg every eight hours on days 1-5 and 15-19) produce overall response rates in 15-20% of patients with complete responses in 4-6% [91-93]. In phase II trials in patients with metastatic melanoma, a high-dose regimen of 600.000 U/Kg IL-2 was administered every eight hours for up to 14 doses; only 16% presented objective response and a small percentage of patients (5%) experienced long-term, durable complete response, which has been interpreted as a potential

cure [84]. However, this therapy has not been shown to improve overall survival in the patient population and has never been evaluated in a phase III setting [94,95]. In addition, IL-2 treatment-related toxicity is severe, which include hemodynamic toxicity (e.g. hypotension, edema, weight gain and decreased renal function), respiratory insufficiency, and neurotoxicity [96,97]. Based on the available data assessing prognostic factors and patient selection, patients with non-visceral metastases and fewer metastatic sites have a much higher response rate. In these select patients, high dose IL-2 may be considered for first-line therapy [98].

2.6.3. *Ipilimumab a new hope for metastatic melanoma*

Stimulation of tumor-expressed Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) with soluble ligands or agonistic mAb triggers the apoptosis [99,100], as well as inhibition of proliferation and secretion of angiogenic cytokines [101]. Preclinical studies showed that blocking CTLA-4 results in anti-tumor activity and tumor regression in many mice tumor models (prostate, breast, lymphoma, melanoma) [102,103]. Two anti-CTLA-4 monoclonal antibodies, generated by different companies, were tested in clinical trials in metastatic melanoma patients: tremelimumab (Pfizer) and ipilimumab/Yervoy (Bristol Myers Squibb), but only the latter was successful in phase III studies. Based on its ability to prolong survival of previously treated as well as untreated metastatic melanoma patients [104,105], ipilimumab received European Union (2010) [106] and FDA (March 2011) approval.

Widely touted as a therapeutic breakthrough, ipilimumab works by enhancing T-cell activity by modifying the function of the CTLA-4 inhibitory receptor. Ipilimumab is directed against the CTLA-4 antigen present on the surface of cytotoxic T-lymphocytes. The presence of the CTLA-4 antigen negatively regulates the activity of T-lymphocytes, ultimately suppressing the immune response [107]. Evidence for ipilimumab offers hope for patients with a clearly lethal disease, but also highlights some of the dangers and relative infancy of immunotherapies in the clinical setting [108].

Blocking the CTLA-4 antigen with ipilimumab will stimulate the patient's own immune response, which will be helpful in destroying cancerous cells [109]. Ipilimumab has been studied in more than 2,000 patients with metastatic melanoma and response patterns showing shrinkage of baseline lesions, a decline of tumor burden with a complete response in few patients. An objective response rate of above 30% was observed in later stages of clinical trials, after failing in the initial stages of the treatment, in which the response rate was 10%. The results of the advanced phase III trials indicate that one third of the patients taking Ipilimumab will receive long-term survival benefits [104]. Objective response rates combining complete response and partial response were in the range of 5–20% [104,110]. Disease control rates were reported averaging 15–30%. In contrast, the two therapies approved by the FDA, high-dose of IL-2 and dacarbazine, are both associated with response rates of only 10 to 20% and a small percentage of complete response. They are not thought to improve overall survival [104]. Two exciting phase III studies tested the clinical effects of Ipilimumab in advanced metastatic melanoma patients. In the first, 676 participants from 125 different medical centers, who had already been given standard treatments, received Ipilimumab or gp100 vaccine separately, or a combination of both, in a randomized, double-blind manner. Treatment with Ipilimumab

improved median overall survival rates (10.0 and 10.1 months in the Ipilimumab-treated groups as compared with 6.4 months in the gp100-only treated group). In the second trial [105], 502 patients who had not previously been treated received either dacarbazine (DTIC, standard care chemotherapy) or Ipilimumab in combination with dacarbazine in a double-blind, placebo-controlled manner. In this experiment, Ipilimumab increased overall survival rates from 9.1 to 11.2 months and 3-year survival from 12.2% to 20.8%. A study of single agent Ipilimumab with intra-patient dose escalation every 2 cycles of therapy also resulted in an increased toxicity with no improvement in the response rates [111]. In this study, patients were initially dosed at 3 mg/kg every three weeks for two doses. If there was no objective response or higher autoimmune toxicity, the dose was increased to 5 mg/kg for two doses and then to 9 mg/kg for two doses. Five out of 46 patients (11%) achieved an objective clinical response at the expense of 35% of patients experiencing significant toxicities. The authors concluded that increasing doses of Ipilimumab to increase autoimmune toxicities did not seem to increase the antitumor activity [111]. Adverse effects, mainly immune related in the skin and gastrointestinal track, were experienced by nearly all patients in the two trials, with about half of the patients suffering from severe adverse effects in the second trial and several severe immune effects-related deaths in the first trial. Therefore, these exciting results also demonstrate the complicity of specifically manipulating immune responses.

3. Multi-drugs combinations

The disappointing results with single agent chemotherapy led to the evaluation of multi-drug combinations regimens in the 1980s in an effort to improve outcome and enhance response rates in patients with metastatic disease. The combinations of different chemotherapeutic drugs) or cytotoxic agents with cytokines occasionally yield substantially higher remission rates without prolonging overall survival [7,21]. The therapeutic schemes that have become established for melanoma are listed in Table 2. The toxicity of combined chemotherapeutics regimes is significantly higher than with monotherapy. Multi-drug combinations can, however, be of palliative use in individual cases and can provide effective treatment for tumor related symptoms. Since regimes are potentially toxic, intensive supportive treatment is crucial for the patient's quality of life [7].

Many of the combination regimens tested in melanoma (see Table 2) have combined dacarbazine with immunologic agents (e.g., IFN, IL-2), hormones (e.g., tamoxifen) or novel biologic agents, each of which individually has shown little single-agent activity. Some of the common combinations of cytotoxic chemotherapeutic regimens used in melanoma are discussed and the few phase III randomized trials that have been published are highlighted.

Historically, promising combination regimens like BOLD (bleomycin, vincristine, lomustine and DTIC) and CVD (cisplatin, vinblastine and DTIC) have induced responses on metastatic lesions in the liver, bone and brain, commonly unresponsive to DTIC alone, even though they have failed to have an impact on patient survival. Several other studies have suggested a significant enhancement of antitumor effect associated with the addition of tamoxifen to

various cytotoxic regimens [112]. The other drug combinations have been observed and some authors recommend the combination of cisplatin, carmustine, dacarbazine and tamoxifen as reference therapy, even though recently presented results of a randomized phase III trial of this combination versus dacarbazine alone show no statistical difference in survival between the two groups. While a survival benefit from dacarbazine based chemotherapy or dacarbazine alone has never been shown in metastatic melanoma patients and, therefore, the survival has remained unchanged over the past 30 years, some long term survivors have been reported after receiving the "Dartmouth regimen" (Dacarbazine /Carmustine /Cisplatin /Tamoxifen) and/or high doses of IL-2 based regimens, whose role is going to be defined in prospective randomized phase III trials [112].

Scheme	Drugs/dosage	Overall response
BHD scheme	BCNU 150 mg/m ² i.v. day 1, only every 2 nd cycle/Hydroxyurea 1500 mg/m ² oral days 1-5/Dacarbazine 150 mg/m ² i.v. days 1-5 every 4 weeks	12.7-30.4%
BOLD scheme	Belomycin 15 mg i.v. days 1 + 4/Vincristine 1 mg/m ² i.v. days 1+ 5/CCNU 80 mg/m ² p.o day 1/Dacarbazine 200 mg/m ² i.v. days 1-5 every 4-6 weeks	22-40%
DVP scheme	Dacarbazine 250 mg/m ² i.v. days 1-5/Vindesine 3 mg/m ² i.v. day 1/Cisplatin 100 mg/m ² i.v. day 1 every 3-4 weeks	31.4-45%
DVP scheme	Dacarbazine 450 mg/m ² i.v. days 1 + 8/Vindesine 3 mg/m ² i.v. day 1 + 8/Cisplatin 50 mg/m ² i.v. day 1 + 8 every 3-4 weeks	24%
CarboTax scheme	Carboplatin AUC6 i.v. day 1, after 4 cycles reduce dose to AUC5/Paclitaxel 225 mg/m ² i.v. day 1 every 3 weeks	12.1% (second-line)
GemTreo scheme	Gemcitabine 1000 mg/m ² i.v. days 1 + 8/Treosulfan 3500 mg/m ² i.v. days 1 + 8 every 4 weeks	33.3% (partial remission and stable disease)
	Carmustine 150 mg/m ² i.v. + Vincristine 2 mg/m ² i.v. on day 1 only	25%
	Cisplatin 20 mg/m ² /day for 4 days starting on day 2 + Vinblastine 1.6 mg/m ² /day x 5 days + Dacarbazine 800 mg/m ² i.v. on day 1	24%
	Tamoxifen 10 mg twice daily by mouth 1 week before chemotherapy + Carmustine 150 mg/m ² on day 1 + Dacarbazine 220 mg/m ² i.v. + Cisplatin 25 mg/m ² /days 1-3	18.5%
	Dacarbazine 250 mg/m ² i.v. days 1-4 every 3 weeks + Detorubicin 120 mg/m ² i.v. every 3 weeks	36%
	Carmustine 150 mg/m ² i.v. on day 1 + Cisplatin 25 mg/m ² i.v./day on days 1-3 + Dacarbazine 220 mg/m ² i.v./day on days 1-3 + Tamoxifen 160 mg orally/day x 7 days prior to chemotherapy. Treatment cycles repeated every 28 days, BCNU every 2 cycles	26%

Scheme	Drugs/dosage	Overall response
	Dacarbazine 2.5 mg/m ² i.v. by means of bolus injection on days 1–4 every 4 weeks + <i>Corynebacterium parvum</i> 7mg i.m. 1 week before starting DTIC and at 4-week intervals thereafter	27%
	Dacarbazine 250 mg/m ² i.v. x 5 days, every 3-weeks + Tamoxifen 20 mg/m ² orally daily	28%
	CCNU 200 mg/m ² once every 6 weeks/Dacarbazine 150 mg/m ² i.v. x 5 days/3 weeks + CCNU 130 mg/m ² 1/6 weeks	15%
	Dacarbazine 250 mg/m ² i.v./day on days 1–5 + Epirubicin 90 mg/m ² on day 1 every 3 weeks	21%
	Vinblastine 6 mg/m ² /day i.v. on days 1–2 + 24-h infusion of Bleomycin 15 units/m ² from days 1–5 + Cisplatin 50 mg/m ² 1 h i.v. infusion on day 5. After four courses, vinblastine and Cisplatin were given alone. Courses repeated on a cycle of 4 weeks	10%
	Dacarbazine 100 mg/m ² 8 h x 6 days every month/ Carmustine 150 mg/m ² + Vincristine 2 mg/m ² every 30 days	24-29%
	Dacarbazine 250 mg/m ² /day i.v. x 5 days every 4 weeks + Vindesine 3 mg/m ² /week	25%
	Dacarbazine 220 mg/m ² on day 1–3 + Carboplatine AUC 5, day 1, 21 Days	21.3%
	IFN- α 15MU/m ² /day i.v. days 1–5 x 3 weeks, then 10MU/m ² s.c. 3x/week + Dacarbazine 200 mg/m ² daily i.v. days 1–5 starting on day 22, every 28 days	21%
	Tamoxifen 20 mg/day v.o. starting day 1 + Dacarbazine 200 mg/m ² /day i.v. days 1–5 every 28 days	18%
	IFN- γ 15MU/m ² /day i.v. days 1–5 x 3 weeks, then 10MU/m ² s.c. 3x/week + orally Tamoxifen 20 mg/day starting day 1 + Dacarbazine 200 mg/m ² /day i.v. days 1–5/28 days	19%
	Dacarbazine 800 mg/m ² i.v. days 1 and 21 + daily INF- α i.m. 3 MIU at days 1–3, 6 MIU days 4–6, and 9 MIU daily thereafter. Started concomitantly	28%
	Dacarbazine 800 mg/m ² i.v. days 1 and 21 + INF- α i.m. 3MIU 3 x /week. Started concomitantly	23%
	Dacarbazine i.v. escalating dose 200 mg/m ² , 400 mg/m ² , 800 mg/m ² /3 weeks; IFN- α s.c. starting at 3 MU/day on days 1–3, 9 MU/day on days 4–70, then 9 MU 3 x /week	21%

BCNU = 1,3-bis(chroethyl)-1-nitrosourea (Carmustine) CCNU = Lomustine

Adapted by Garbe et al. (2008) [21] and Mouawad et al. (2010) [7].

Table 2. Multi-drug combinations and their activity in melanoma.

The Dartmouth Regimen was described for the first time in 1984 with the treatment of 20 patients with melanoma. This association showed promising results, with high response rates of 40% to 50% [113,114]. However, another study shows a response rate of 18.5% with this combination [115]. In another randomized phase II trial, Dartmouth Regimen was compared with dacarbazine alone in patients with melanoma, wherein 60 patients were randomly assigned to receive carmustine 150 mg/m² intravenously on day one, cisplatin 25 mg/m² intravenously daily on days one to three, dacarbazine 220 mg/m² intravenously daily on days one to three, and tamoxifen 160 mg orally daily for seven days. The overall response was 26%, complete responses were 2.5%, with median progression-free survival and the median survival four and nine months respectively. These results show that the combination had limited impact on overall survival when compared with dacarbazine alone, with the additional drawback of high toxicity [116].

In a multicenter phase III trial, 240 patients with measurable stage IV melanoma were randomly selected to receive the Dartmouth regimen (dacarbazine 220 mg/m² and cisplatin 25 mg/m² on days one to three, carmustine 150 mg/m² on day one every other cycle and tamoxifen 10 mg orally bid). The treatment was repeated every three weeks. The results showed median survival time from randomization of seven months; response rate was 18.5% when compared with dacarbazine alone this treatment was not better [115]. In addition to the low activity, the Dartmouth Regimen showed high toxicity, mainly bone marrow suppression, nausea, vomiting and fatigue. Although advanced melanoma is often not curable with systemic therapy, the considered use of currently available regimens can induce clinically significant remissions and, possibly, prolong the life of some patients.

Su et al. (2011) [124] evaluated the combination of carmustine (150 mg/m², on day one every eight weeks), cisplatin (25 mg/m², days one to three every four weeks), dacarbazine (220 mg/m² days one to three every four weeks), tamoxifen (10 mg twice a day) and IL-2 18 Million International Units in divided doses by subcutaneous injection three times a week for four weeks. The treatment was repeated for a total of six cycles or until disease progression or unbearable toxicity. The response rate was 32.5% including 5% with a complete response, 27.5% with a partial response and 17.5% with a stable disease. The median progression-free survival was 6.2 months and median overall survival was 11.3 months. The toxicity associated with IL-2 included indurate injection site, fever, chills, itchy skin, bone pain and myalgia and eosinophilia.

The Dartmouth Regimen was associated with IL-2 and IFN- α . In those patients who received sequential immunotherapy, each cycle of chemotherapy was followed by outpatient s.c. IL-2 (10 x 10⁶ IU/m², on days three to five, week four; 5 x 10⁶ IU/m², on days one, three and five, week five) and s.c. IFN- α (5 x 10⁶ IU/m², on day one, week four; on days one, three and five, week five). The overall response rate of patients treated with the combination of chemotherapy and IL-2/IFN- α was 34.3% with complete responses (10.9%) and partial responses (23.4%). In patients treated with chemotherapy only, the overall response rate was 29.9% with complete responses (13.3%) and partial responses (16.6%). There was no significant difference in median progression-free survival (zero months vs. four months) and in median overall survival (12

months vs. 13 months) for combined chemoimmunotherapy and for chemotherapy, respectively [117].

A prospective randomized phase III clinical trial was performed to assess whether the addition of tamoxifen to the Dartmouth Regimen would ameliorate the responses. The median survival observed was 6.9 months, but the progression-free survival as well as the overall survival was not different from the treatment with Dartmouth Regimen alone [118].

Other chemotherapeutic agents have also been combined with tamoxifen for the treatment of metastatic melanoma. Bajetta et al. (1993) [119] treated two groups of patients sequentially; the first group received carboplatin and cytarabine alone and for the second group, tamoxifen 40 mg was added daily throughout the cycle. Of 21 evaluable patients in the first group, 19% responded. In contrast, 8% of patients responded in the tamoxifen-treated group. All patients had received prior chemotherapy and/or immunotherapy. In an attempt to confirm synergism between tamoxifen and cisplatin and to overcome clinically established cisplatin resistance, McClay et al. (1995) [120] treated 24 metastatic melanoma patients with cisplatin 100 mg/m² every three weeks; 13% of patients responded. Those who failed to respond clinically (cisplatin resistant) were then treated with the same dose of cisplatin plus tamoxifen 160 mg on day one, followed by 20 mg daily throughout the three-week cycle. In 19 assessable patients, 16% achieved a complete response or partial response as conventionally defined; however, when the authors included mixed responses, the overall response rate (32%) was statistically significantly. In a phase I study, McClay et al. (1995) [120] treated successive groups of patients with escalating doses of tamoxifen and weekly cisplatin 80 mg/m². Patients received up to 320 mg/m² at the time of the report and none of the patients responded at doses less than 240 mg/m².

Studies have combined chemotherapy, immunomodulators and tamoxifen. Antoine et al. (1995) [121] added tamoxifen to a regimen of cisplatin, IL-2, and IFN- γ . The response rate with tamoxifen (41%) was lower than the response rate observed without tamoxifen (54%). Rixe et al. (1994) [122] treated 91 patients with high-dose cisplatin, IL-2, IFN- α and tamoxifen. The trial was stopped prematurely due to an increased frequency of sepsis and because the response rates were similar to those observed in historical controls that did not receive tamoxifen.

Fierro et al. (1993) [123] treated 32 patients with a combination including carmustine, cisplatin, dacarbazine and tamoxifen, which showed an overall response rate of 47%, complete response of 16%, partial response of 31% and median survival of 10 months. The pretreatment with INF- α did not modify the survival or the response rate.

In a phase II trial with a high dose of tamoxifen and cisplatin administered weekly were used; tamoxifen was started on day one with a dose of 240 mg/day. Cisplatin (80 mg/m²) was started on day two and repeated weekly for a total of three weeks. During week four, the patient was not treated with cisplatin but was evaluated for response. If disease stabilization or regression was documented, the patient received a second three-week cycle of cisplatin and was then re-evaluated for response. Patients with progressive disease at any evaluation were removed from the study. In 28 consecutive patients, the overall response rate was 32%. Toxicity was

primarily nausea and vomiting. While this combination presented activity, the overall response rate was not significantly better than that observed with the original Dartmouth regimen and besides, the toxicity is substantial [128].

The cisplatin, vinblastine and bleomycin were combined in the treatment of 51 patients with metastatic melanoma; among these patients only 22% had complete or partial response. However, the response durations were brief and the toxicity was substantial. The authors concluded that such a combination was not sufficiently beneficial to warrant its use in metastatic melanoma [132]. Bajetta et al. (1982) [133] treated 22 patients with metastatic melanoma with vinblastine, bleomycin and cisplatin. In this study, the survival rate was 10 months and the results showed that this combination did not exceed the treatment with dacarbazine. Nathanson et al. (1981) [134] also studied this combination and the patients showed a median duration of response of 26 weeks associated with significant toxicity, marrow suppressive, gastrointestinal, pulmonary and mucocutaneous.

The BOLD scheme (bleomycin, vincristine, lomustine plus dacarbazine) included bleomycin at 7.5 units subcutaneously in the first course and 15 units in subsequent courses on days one and four; vincristine at 1mg/m² intravenously on days one and five; lomustine at 80 mg/m² on day one and dacarbazine 200 mg/m² intravenously on days one through five. These treatments showed 9% complete response, 31% partial response and 17% stabilization of disease and overall median survival of 31 weeks. The authors concluded that the BOLD scheme was an effective alternative treatment for metastatic melanoma, because the overall toxicity was moderate [135]. Several other works that used the BOLD scheme in combination with IFN- α showed better results with response rates from 13 to 24%, which is not significant when compared with the treatment with the BOLD scheme alone, but patients with soft tissue or lung metastases may achieve more complete response [124]. Another study reported the treatment of twenty-two patients with metastatic melanoma with the BOLD scheme every four weeks, together with IFN- α 3 x 10⁶ IU daily for six weeks, followed by 6 x 10⁶ IU three times per week. This treatment showed median progression-free survival of four months and median overall survival of 12 months, associated with moderate toxicity [137]. A similar scheme was used in a study reported by Atzpodien et al. (2002) [125], in which the median progression-free survival was 1.9 months and overall survival 10.6 months, but the scheme showed more toxicity in 13% of the patients (alopecia and neurotoxicity). The regimen of BOLD plus interferon is active in the treatment of metastatic uveal melanoma. The precise role of the regimen has to be defined in light of its toxicity, particularly the unpredictable pulmonary toxicity. The pattern of occurrence of these pulmonary events is most consistent with either an acquired hypersensitivity reaction, or a cumulative toxic effect from two or more agents. Patients considered for treatment with this regimen must be judiciously selected. Those with no clear contraindications may benefit from a trial of this regimen, but they must be monitored closely.

Another regimen that has shown good results was the combination of bleomycin 15 mg administered subcutaneously on days one and four, vindesine 3 mg/m² administered intravenously on days one and five and lomustine 80 mg/m² orally on day one and dacarbazine

200 mg/m² intravenously on days one through five in a phase III study. This combined response rate of 45% is considerably better than that seen in single- or dual-agent chemotherapy. The toxicity was tolerable. The median survival for all treated patients was 43 weeks, and the median follow-up time was now 63 weeks [139]. Stables et al. (1992) [140] evaluated the treatment of 72 patients with melanoma with a regime combination including bleomycin, vindesine, lomustine and dacarbazine. This study showed a complete response in 17.6% of the patients, partial response in 14.7% of the patients and overall median survival of 38 weeks. These results are comparable with other combinations of chemotherapy regimens, which have not yet been supplanted by the newer biological therapies.

Based on the independent activity of cisplatin, vinblastine, and dacarbazine (CVD), a combination of these agents was used in the treatment of patients with advanced melanoma. Different doses are used in this treatment scheme, but the most common combination is vinblastine in a dose of 1.6 mg/m²/d for five days, dacarbazine in a dose of 800 mg/m² intravenously on day one, and cisplatin in a dose of 20 mg/m²/d for four days starting on day two of chemotherapy or, 3-week cycles of cisplatin 20 mg/m²/day × 4; vinblastine 2 mg/m²/day × 4, and dacarbazine 800 mg/m² on day one [141]. In a phase II trial with 50 evaluable patients, a response rate of 40% was achieved with an estimated 1-year survival of 50% [141]. In a randomized trial using biochemotherapy, in which CVD was the control arm, the same investigators reported that CVD showed an objective response rate of 27% and an estimated 1-year survival of approximately 40% [142]. The median duration of the response was nine months and the median survival time of the responders was 12 months. The treatment was associated with significant toxicity consisting of nausea, vomiting, diarrhoea and partial hair loss. Additionally, neutropenia with a median nadir granulocyte count of 500/microliters was observed, and significant anaemia required blood transfusions in a majority of the patients after three to four courses of chemotherapy. The dose-limiting toxicity was peripheral neuropathy, which required discontinuation of cisplatin after six to eight courses of chemotherapy [141].

Another study used a combination of dacarbazine 250 mg/m²/day one to three, cisplatin 30 mg/m²/day i.v. on days one to three and IFN- α 10 × 10⁶ U/m²/day subcutaneously on days one to five, and one group received the combination mentioned, plus IL-2 as a continuous i.v. infusion in a decreasing schedule starting on day 5 with 18 million U/m² over 6 hours followed by 18 million U/m² over 12 hours, 18 million U/m² over 24 hours, and a maintenance dose of 4.5 million U/m²/24 hours for an additional 72 hours. Cycles were repeated every four weeks for a maximum of four cycles. This study showed that the combination was not better than the CVD combination, and was associated with low efficacy and higher toxicity. The authors concluded that the combination neither conferred a clinically meaningful survival benefit for most patients with advanced melanoma, nor conferred an increase in rate response or progression time [94].

Another study has shown results related to the treatment of patients with metastatic melanoma with six concurrent biochemotherapy dose levels. The doses were programmed as follows: dacarbazine 800 mg/m² or 1000 mg/m² (Day 1); cisplatin 25 mg/m² or 30 mg/m² (Days 1-4); vinblastine 1.6 mg/m² or 1.8 mg/m² (Days 1-5); interleukin-2 9 million units (MU) per m² or 12

MU/m² as a 24 h continuous infusion (Days 1-4); and interferon-alpha-2b 5 MU/m², 10 MU/m², or 15 MU/m² (Days 1-5) and 5 MU/m² (Days 7, 9, and 11) administered subcutaneously. The dose of IFN- α was reduced in some patients because of thrombocytopenia after five days, and other toxic effects observed included encephalopathy, renal and hepatic dysfunction, pancreatitis and ileus. This treatment showed a median time to disease progression of 6.9 months, and median survival duration of 12.2 months [143]. In a study reported by Legha et al. (1996) [90] the treatment with CVD plus IL-2 or IFN- α reached an overall response rate of 60%, although the duration of partial response was short (median eight months), the median survival of patients receiving sequential biochemotherapy was 13 months compared to nine months observed in the CVD treated group. The treatment with biochemotherapy was associated with severe toxicity including intense myelosuppression, infections; IL-2 induced constitutional toxicity and hypotension. The biochemotherapy regimen produced an apparent increase in the median survival compared to that observed with the CVD regimen.

In a randomized study, 176 patients received cisplatin and dacarbazine with or without carmustine every 21 days, or the same regimen followed by low-dose subcutaneous IL-2 for eight days and IFN- α three times a week, both for six cycles. The regimen without IL-2 and IFN- α resulted in a median overall survival of 9.5 months and the regimen with IL-2 and IFN- α of 11.0 months. The regimen with low-dose immunotherapy did not produce a statistically significant advantage in overall survival, time to progression or overall response when compared with the regimen without immunotherapy. However, the results were not different from the treatment with high dose IL-2 [87].

In a phase III study, 326 patients with metastatic melanoma received the treatment consisting of a combination of dacarbazine (250 mg/m² intravenously days one to five every four weeks) and vindesine (3 mg/m² intravenously day one weekly), with or without the addition of cisplatin (100 mg/m² intravenously day one every four weeks). In this study the authors did not observe a significant difference in overall survival with addition of cisplatin, but the median time to progression was significantly longer in patients treated with dacarbazine, vindesine and cisplatin than patients that did not receive cisplatin (4.2 versus 2.2 months respectively). However, the combination of the three drugs did not change overall survival but did significantly increase toxicity such as leukopenia, alopecia and nausea/vomiting [144]. Similar treatment was used in a phase II study with forty patients with disseminated malignant melanoma. In this study, the authors observed a 38% response rate and median response duration of four months, but the toxicity was intolerable with symptoms such as nephrotoxicity, ototoxicity, hypotonia and nausea/vomiting [126].

Fotemustine has also been used in combination with dacarbazine, in patients with metastatic melanoma. The response rate was 27.2%, confirming the activity of fotemustine. The combination of two drugs showed significant toxicity, mainly haematological with leukopenia and thrombocytopenia. This schedule with sequential dacarbazine and fotemustine had low activity against metastatic melanoma, and the response rate for cerebral metastases was not superior to that shown in other studies with single agent fotemustine, however, the treatment was well tolerated and can be prescribed to outpatients [54].

Preclinical studies have shown some clinical activity against melanoma. It consisted of a combination of carboplatin at an area under the curve (AUC) of 7.5 and paclitaxel at 175 mg/m² over three hours administered to 17 patients [127]. There was a 20% response rate with three partial responders in the 15 evaluable patients, with a median survival of nine months. Another phase II study showed results with the administration of paclitaxel either as monotherapy or combined with carboplatin. In the first treatment paclitaxel was administered at a dose of 100 mg/m² intravenously on day one each week for six weeks; in the second treatment paclitaxel was administered at a dose of 80 mg/m² intravenously followed by carboplatin 200 mg/m² on day one each week for six weeks. The next cycle was administered after a two-week intermission. This study showed median survival time of 209 days in patients that received only paclitaxel, and 218 days for those treated with paclitaxel/carboplatin, with overall response rates of less than 10% for both treatments [68]. Paclitaxel at a dose of 100 mg/m² and carboplatin of 2 AUC was administered on days one, eight and 15 of a 28 day cycle. This treatment showed 26% partial response and 19% stable disease and a median overall survival of 7.8 months [128].

Kottschade et al. (2011) [148] conducted a study in which the patients were treated with paclitaxel 100 mg/m² and carboplatin AUC six administered on days one, eight and 15 every 28 days. The median number of treatment cycles was four. This treatment showed 25.6% responses and median overall survival of 11.1 months. In a study conducted by Pflugfelder et al. (2011) [149], patients received intravenous paclitaxel 225 mg/m² plus intravenous carboplatin AUC 6 on day one of a 21 day cycle, with a dose reduction after the fourth cycle to carboplatin AUC five and paclitaxel 175 mg/m². This study showed median progression-free survival of 10 weeks and median overall survival of 31 weeks. Response, progression-free, and overall survival were equivalent in first and second line patients. Sixty patients out of 61 died after a median follow-up of seven months. This treatment showed, in all studies, severe toxicities including neutropenia, thrombocytopenia, neurosensory problems, fatigue, nausea and vomiting [68,127,129].

The development of targeted therapies has provided new options for the management of patients with advanced melanoma. There has been particular interest in agents that target the mitogen-activated protein kinase pathway, which controls tumor growth and survival, and promotes angiogenesis. Recently, sorafenib, an oral multikinase inhibitor has been tested in combination with carboplatin and paclitaxel. A phase I trial conducted with 38 patients, who received either 100, 200, or 400 mg of sorafenib twice daily on days 2 to 19 of a 21-day cycle with carboplatin at AUC 6 and paclitaxel at 225 mg/m² administered on day one [130]. The overall response was 10 out of 24 treated patients with one complete response. Another study, however, showed that the treatment of 270 patients with paclitaxel at 225 mg/m² and carboplatin at AUC 6 once every three weeks with or without sorafenib at 400 mg twice daily on days two to 19. There was no difference in progression-free survival, which was the primary endpoint, or in response rate [131]. The control group (no sorafenib) showed a response rate of 11% with a median progression-free survival of 17.4 weeks; the median overall survival was 42 weeks. The cohort receiving sorafenib had essentially identical outcomes. The conclusion

was that the addition of sorafenib to carboplatin/paclitaxel did not improve the response rate in contrast to the original observations in the phase I trial [130].

Recently, patients were randomly assigned in a two-to-one ratio to carboplatin (AUC, 5) plus paclitaxel (175 mg/m²) and bevacizumab (15 mg/kg) administered intravenously once every three weeks. This study showed an overall response rate of 25.5%. The study did not meet the primary objective of statistically significant improvement in median survival with the addition of bevacizumab to carboplatin plus paclitaxel. A larger phase III study will be necessary to determine whether there is any benefit to the addition of bevacizumab to carboplatin plus paclitaxel in this disease setting [132].

Schmittl et al. (2006) [133] compared the combination of gemcitabine plus treosulfan with treosulfan alone in patients with metastatic melanoma; the patients received 1000 mg/m² of gemcitabine plus 3500 mg/m² of treosulfan or 3500 mg/m² of treosulfan on days one and eight, and in both groups the cycle was repeated on day 29. Median progression-free survival was three months and two months for patients undergoing the treatment with gemcitabine plus treosulfan and treosulfan, respectively. In a phase II clinical trial, gemcitabine plus treosulfan were tested in patients with metastatic melanoma. The patients received 1000 mg/m² of gemcitabine and treosulfan at a dose of 2500 or 3000 mg/m² in group one, and 3500 or 4000 mg/m² in group two on days one and eight every four weeks. In the group of patients that were treated with treosulfan in a dose less or equal to 3000 mg/m² the response rate was not observed. Among the patients treated with a dose equal to or more than 3500 mg/m² a 5% partial remission was observed, with median survival time of nine months [134]. Another study by Schmittl et al. (2005) [154] combined gemcitabine plus treosulfan and cisplatin, in which the patients received 30 or 40 mg/m² of cisplatin, 1000 mg/m² of gemcitabine and 3000 mg/m² of treosulfan on days one and eight repeated on day 29; the median overall survival was 7.7 months, associated with excessive haematological toxicity.

Dacarbazine 250 mg/m²/day was combined with epirubicin 90 mg/m² on day one every three weeks. Partial response of 21.1% was observed [135].

Another phase III study of ipilimumab was carried out in patients with previously untreated metastatic melanoma. In this case, ipilimumab in combination with dacarbazine was compared with dacarbazine plus placebo, showing improved overall survival with the antibody therapy. Responses with the combined therapies seemed to be higher than the therapies with a single agent (17% compared to 5%) [105]. Data from preclinical and clinical studies have shown that ipilimumab can cause tumor regression in patients with metastatic melanoma with response rates of 5.8-22%. Phase III trials have demonstrated a benefit to median overall survival in the first-line setting in combination with dacarbazine versus dacarbazine alone (11.2 versus 9.1 months), and in the second-line setting in combination with glycoprotein 100 peptide vaccine (gp100) vs. gp100 alone (10.1 versus 6.4 months). The main toxicities of ipilimumab are immune related, most commonly skin and gastrointestinal troubles. Bowel perforation and treatment-related deaths have occurred, although prompt use of steroids and other immunosuppressive agents can minimize these risks [136].

In phase II study patients with metastatic melanoma received ipilimumab at three mg/kg every four weeks for four doses, either alone or with up to six-five-day courses of dacarbazine 250 mg/m²/day. The response rate was 14.3% with ipilimumab plus dacarbazine and median overall survival was 14.3 months [137].

4. News strategies for treatment of metastatic melanoma

4.1. Signalling molecules targeted therapy

One of the most common signalling pathways affected by mutations in melanoma is the RAF/MEK/ERK pathway, a highly conserved group of proteins that regulate cell growth, division and death. In melanoma, the most common mutations are in proteins like BRAF, NRAS, HRAS, and KIT and lead to constitutive activation of the RAF/MEK/ERK pathway, which stimulates pro-proliferative genes. BRAF, the serine/threonine specific protein kinase, is triggered by somatic mutations in 50–70% of melanoma cases. A substitution of glutamic acid for valine at codon 600 (V600E) is the most common BRAF mutation known [138]. Recently, genotype-selected metastatic melanoma patients with positive BRAF mutation have been submitted to clinical trials with drabafenib (accelerated dose titration with 12 mg initial dose), an inhibitor of BRAF kinase, selective for mutations in BRAF. High response rates were achieved in patients with melanoma brain metastases leading to nine out of ten patients with a size reduction of brain lesions [139]. A phase III trial comparing drabafenib (150 mg twice daily, orally) with dacarbazine (1000 mg/m² intravenously every 3 weeks), higher median progression-free survival was observed in the drabafenib group than those for dacarbazine. However, dose reduction of drabafenib was needed in 28% of patients due to adverse effects including nausea, vomiting, fatigue and neutropenia. Dose reduction was also necessary in patients receiving dacarbazine (17%) [131].

Despite these front line studies that validated BRAF inhibition and genotyping analysis of BRAF mutational status as a feasible tool in patients undergoing clinical trials with signalling molecules targeted therapy, side effects remain the most important concern during therapy. In this field, another BRAF inhibitor, vemurafenib, was compared to dacarbazine, and studies demonstrated that vemurafenib-treated patients had higher overall survival rate and final analysis for progression-free survival than those who received dacarbazine. Also, the vemurafenib-treated group had relative reduction of 63% in the risk of death and of 74% in the risk of either death or disease progression, as compared to dacarbazine. Regarding adverse effects, 18% of patients treated with vemurafenib presented cutaneous squamous-cell carcinoma, keratoacanthoma, or both [10].

However, after genetic status assessment, Falchook et al. (2012) [140] demonstrated that trametinib, a reversible selective allosteric inhibitor of MEK1 and MEK2, had a good clinical activity in patients with melanoma, suggesting that MEK could be a valid therapeutic target in BRAF-mutant melanoma. Interestingly, patients were free of proliferative skin lesions such as squamous-cell carcinoma, a common adverse effect associated with BRAF inhibitors, representing an advantage of trametinib in treating early-stage diseases [140].

With regard to drug resistance, antiapoptotic proteins of the Bcl-2 family are closely involved in this process. This group of proteins regulates apoptosis by blocking the release of cytochrome *c* and the overexpression of Bcl-2, which has been partially associated with drug resistance phenomena (Soengas et al., 2003). In this field, antisense Bcl-2 therapy may be an alternative adjuvant therapy in melanoma clinical trials. In the past, *in vitro* experimental transfection of Bcl-2 was performed in both haematological and solid tumor cells, leading to multidrug resistant phenotype [141] and recently, oblimersen sodium was combined with dacarbazine in a clinical trial involving 771 patients with advanced melanoma [13]. This new drug decreased the expression of Bcl-2 protein by increasing the cleavage of Bcl-2 mRNA by RNase H [142,143].

Another mechanism of tumor resistance is related to the repair process of damaged DNA. The poly (ADP-ribose) polymerase-1 (PARP) is the nuclear key enzyme of this process and mediates the recruitment and activation of repair factors in the DNA break leading to base excision repair [144]. The first phase I clinical trial with inhibitor of PARP was conducted with AG014699, a prodrug of AG014447, a potent inhibitor of PARP, combined with temozolomide in patients with solid tumors and metastatic melanoma. In this clinical trial, AG014699 was well tolerated and proved to be highly and selectively toxic to cells that underwent DNA repair [145].

Finally, one of the most studied signalling molecules is NF- κ B, a pathway that melanoma cells use to increase survival, proliferation and resistance to apoptosis, leading to progression of tumor and metastasis appearance [146,147]. One of the first NF- κ B inhibitors used in clinical trials was bortezomib. Combined with temozolomide, a phase I clinical trial was conducted in order to establish a schedule use of these drugs, define phase II doses and assess the NF- κ B activity in blood. Results demonstrated that bortezomib failed to inhibit NF- κ B, and toxicities including neurotoxicity, fatigue, diarrhoea and rash were reported [18]. Furthermore, two years later another phase I trial with patients with advanced solid tumors was conducted in order to establish new maximum tolerated doses for the combination of bortezomib and temozolomide with inconclusive results [148]. In addition, both groups of authors suggested that new studies with strong preclinical rationale have to be conducted in order to cover all the changes undergone at tumor sites [18,148].

4.2. Electrochemotherapy

After years of experience in delivering genes, DNA and drugs into mammalian cells using electroporation [149,150], a local tumor ablation modality, the electrochemotherapy (ECT) has been used effectively in the treatment of various solid tumors, particularly melanoma. ECT combines chemotherapy and electroporation to increase drug uptake into cancer cells [151]. The European Standard Operating Procedures of Electrochemotherapy (ESOPE) was the largest study reported so far in patients with melanoma [152]. In this huge clinical trial, the efficacy and safety of ECT were evaluated according to the drug used, route of administration and types of electrodes for electroporation. The ESOPE project consisted of the administration of bleomycin (ineffective for systemic conventional therapy), both intravenously (15000 IU/m²) and intratumorally (1000 IU/cm³), followed by electroportation. Cisplatin was also

administered in some patients (only intratumorally), because previous reports had not substantiated the high efficacy of electrochemotherapy using intravenous injection of cisplatin [153]. Almost 74% of patients had complete response rate achieved on the electrochemotherapy treated tumor nodules. Furthermore, side effects were minor and acceptable by patients and showed great advantages regarding cost and effectiveness.

Recently, another approach using ECT, based on the ESOPE study, has been conducted in patients with cutaneous and subcutaneous melanoma metastases. Bleomycin was also used as a drug of choice for most cases, and ECT was conducted after intravenous administration of bleomycin (15 mg/m²). Results demonstrated that 62% of patients had an objective response rate for all treated metastases. The authors also observed that 23% of patients had complete response of metastatic nodules and only 8% of patients showed disease progression. Because ECT was achieved using low doses of bleomycin, no systemic side effects were observed [154].

Regarding disseminated superficial metastasis, Campana et al. (2012) [175] demonstrated that several ECT cycles on metastases, unsuitable for resection or unresponsive to conventional chemotherapy, were effective only in patients with few and small metastases on the lower limb. Besides the restricted response, ECT was well tolerated and side effects were limited.

4.3. Transarterial chemoembolization

In an attempt to manage metastatic melanoma, especially liver metastases, new approaches have been tested using the dual blood vessels supply of the liver in combination with anti-cancer drugs such as irinotecan and cisplatin. This new approach, the so-called transarterial chemoembolization (TACE), takes therapeutic advantage of the hepatic artery and branches of the portal vein. By infusing chemotherapeutic agents directly into vessels followed by infusion of embolic microspheres, localized therapy and the concentration of the drug in tumor are achieved. In this field, Venturini et al. (2012) [155] enrolled five chemotherapy-naive patients with liver metastasis of uveal melanoma in an irinotecan-eluting chemoembolization trial. TACE was successfully achieved and well-tolerated in all five patients. In addition, a response rate of 80% was achieved in relation to lesion size and remission. Apart from local pain and one isolated case of cholecystitis, no further side effects were observed.

In a more complex study design including cisplatin, carboplatin, fotemustine or melphalan infusion followed by polyvinyl alcohol particles for embolization, 11 patients were monitored for five to 58 months, and 57% of them presented partial response compared to 29% with stable disease, and 14% presented new lesions detected during the follow up period. Median survival of all patients was 11.5 months after metastasis diagnosis [156]. In addition, abdominal and local pain seems to be the most common effect after TACE procedures. Huppert et al. (2010) [177] reported that pain began 1-4h after embolization and lasted around one to three days, and that morphine was necessary in some cases.

Transarterial chemoembolization has the advantages of reducing systemic toxicity of drugs and increasing intratumor effects leading to better results. However, Schusser et al. (2010) [178] also reported serious side effects after TACE protocol using fotemustin (100 mg/m²) as a

first choice, followed by cisplatin (50 mg), including splenic infarction, thrombocytopenia and gastric ulcer; all events required conservative treatment and prolonged hospitalization.

4.4. Adoptive T cells therapy

Immunotherapy has been widely reported as a promising alternative for a variety of malignancies including melanoma [157]. To divert the majority of the immune cells to react against tumor has been a huge challenge among physicians. Particularly, adoptive T cells (ATC) optimally expanded ex-vivo, in theory, could enhance anti-tumor immune response and form memory protection against recurrence. A well-established expansion of tumor-infiltrating lymphocytes (TIL) has been carefully used in clinical trials, especially in metastatic melanoma patients [79,158-160]. Recently, 93 patients with metastatic melanoma were treated with the ATC transfer of autologous TIL and IL-2 with a median follow-up of 62 months. 22% of patients showed complete regression and durable complete responses were seen in patients who had a median of three different organ sites of metastases including lung, liver, adrenal, muscle, lymphonodes, and skin [161]. However, some limitation was observed in ATC therapy. Joseph et al. (2011) [184] reported a negative influence in initial TIL outgrowth in samples obtained from patients who received systemic therapy 30 days before tumor harvest. This data suggested that parameters like prior systemic chemotherapy should be considered as important criteria for patients undergoing ATC therapy. The success of autologous TIL derived from metastatic tumor tissue is dependent on the TIL initial outgrowth and the tumor characteristics, rate of TIL and parallel systemic chemotherapy, because the latter determines the rate of TIL harvested [162].

In an attempt to make up for some limitations of ATC therapy, especially those related to TIL harvested outgrowth and based on the observation that melanoma-reactive TIL could be generated from only 50% of harvested samples [163], autologous T cells transduction with T-cell receptors (TCR) against the antigen NY-ESO-1 was carried out. A retroviral vector encoding a TCR, which recognizes the peptide NY-ESO-1, an antigen highly expressed in almost 50% of melanoma metastases, was transfected into T-cells of patients, cultured ex-vivo and transferred to patients together with HD IL-2, as a complementary therapy. Results from this vanguard clinical trial showed that five of the 11 patients with metastatic melanoma experienced an objective response including two complete responses during the follow-up period (20 months), and 2 patients demonstrated complete regressions that persisted after one year.

5. Insights in side effects prevention

It is clear that the cure for malignant melanoma is still a challenge. One can conceive of many strategies to combat the disease: i) prevention ii) several kinds of therapies, iii) amelioration of side effects caused by the therapies. In this regard, there is evidence that natural or semi-synthetic compounds can feasibly allow for a wide variety of potential drugs to be employed in a multitarget approach alone and/or in combined therapies.

Syed and Muktar (2011) [164] summarized some options in a review article about several plant products for the prevention and treatment of different kinds of cancer, including melanoma. Among the compounds they mentioned: genistein, epigallocatechin gallate, resveratrol, curcumin, fisetin, silymarin lupeol, which are able to stop the growth of tumor cells through their own multitarget drug properties [164]. In the same context, several research groups, including ours, have studied gallic acid and its ester derivatives [165-168]. It has been shown that gallic acid and gallates differing only in the number of carbon atoms in the lateral chain present antitumor properties, whose mechanism of action is also by a multitarget way, including cell growth and metastasis inhibition, action against the drug efflux, helping to avoid the development of resistance as well as a selective cytotoxicity

6. Conclusion

Significant advancements have been made in the last few years about melanoma treatment, including important information on the molecular mechanism of action of the drugs and the signalling pathways related to the disease. However, the treatment of patients with advanced melanoma remains unsatisfactory. Single agent or combinations of chemotherapies including new agents or biologic response modifiers have not resulted in response rates of durable remissions high enough to affect median survival. The approval by the FDA of ipilimumab in 2011 brought some optimism among the clinicians who treat patients with metastatic melanoma, but this therapy also has its limitations.

Promising sources of alternative molecules for cancer seem to be plants. In general, natural or semi-synthetic compounds have shown effectiveness not only in the prevention of the disease and the development of resistance, but also in important antitumoral activities in vitro and in pre-clinical assays.

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