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# Therapeutic Agents for Advanced Melanoma

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Zhao Wang, Wei Li and Duane D. Miller

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## 1. Introduction

Melanoma is an extremely complicated disease, with many gene mutations and alternations in signaling pathways. Because effective treatment for melanoma is lacking, the prognosis for metastatic melanoma patients remains very poor. Over the past 30 years, significant efforts have been made to search for better agents or strategies to fight this deadly disease. Numerous clinical trials at different stages have been carried out. Although most of them have failed, some did show very promising results. New treatment strategies have resulted in paradigm shift in our approach to melanoma therapy. These shining examples may markedly change our philosophy about melanoma treatment.

The year 2011 marked a fruitful year for melanoma research. The FDA approved three drugs for advanced melanoma treatment: ipilimumab (an anti-cytotoxic T lymphocyte antigen-4 monoclonal antibody), vemurafenib (a selective BRAF inhibitor), and pegylated interferon- $\alpha$ 2b for adjuvant setting usage [1]. However, there are still significant limitations for melanoma treatment. Ipilimumab only prolonged the survival time for metastatic melanoma patient from an average of 6.5 months to an average of 10 months. This treatment also has been associated with strong immunological adverse effects; severe to fatal autoimmune reactions were seen in 12.9% of patients treated with ipilimumab in a clinical trial that enrolled 676 melanoma patients [2]. Vemurafenib is not effective for melanoma patients with wide type BRAF, and confirmation of BRAFV600E mutation-positive melanoma using an FDA-approved test is required before treatment with vemurafenib. This treatment also only prolonged the median survival time for advanced melanoma patients 2~3 months [1]. More importantly, despite the high initial response rate for patients with BRAFV600E mutation to vemurafenib, virtually all the patients developed primary or acquired resistance to this drug in the end [3]. With the rapidly rising incidents of this disease and the high resistance to current therapeutic agents, developing more effective drugs for melanoma is very important.

In this chapter, we will review available therapeutic agents for advanced melanoma as well as agents that are still under clinical development. We will focus on their mechanism of action, development history, and therapeutic effects. In learning from our efforts in the past, we must continue to challenge the current paradigms of treatment as we forge new paths to more effective treatment options. This will likely involve a multimodal approach to therapy utilizing all of the available tools in our arsenal.

## 2. Chemotherapy agents

According to the current 7<sup>th</sup> edition American Joint Committee of Cancer (AJCC) staging system, melanoma can be pathologically classified in the following stages: 0, IA, IB, IIA, IIB, IIC, IIIA, IIIB, IIIC, and IV. Stage 0 is in situ melanoma. Stage I and II are growth phase of localized cutaneous melanoma with increasing thickness. Stage III has regional involvement of lymph node. Stage IV means distant metastasis. Normally stage III and IV melanoma are called metastatic melanoma. Localized melanoma is curable with complete surgical excision in most patients. But currently treatment for metastatic melanoma is still very challenging. Only two chemotherapy drugs in current use have been approved by the Food and Drug Administration (FDA) for metastatic melanoma: dacarbazine (DTIC) and vemurafenib. Other agents that have been tried on melanoma patients are also discussed below.

### 2.1. Dacarbazine (DTIC)

DTIC is the first FDA approved chemotherapy drug for metastatic melanoma. This drug gained FDA approval in May 1975 as DTIC-Dome for the treatment of metastatic melanoma. It was initially marketed by Bayer. The therapeutic effect of DTIC is believed to be produced through alkylation of DNA. While its anticancer mechanism of action is still not fully understood, DTIC is believed to be first metabolically bioactivated through a series of reactions involving CYP450. Initial demethylation to MTIC [3-methyl-(triazene-1-yl)imidazole-4-carboxamide] is followed by formation of diazomethane, the active moiety of DTIC and a potent methylating agent [4].

DTIC has produced response rates of from 15% to 25% in single-institution trials. But the overall response rate has fallen over the years from 15% to 7% and less than 5% of responses are complete in phase 3 trials. The median response durations to DTIC are 5 to 6 months. Long-term follow-up of patients treated with DTIC alone shows that only <2% can be anticipated to survive for more than 6 years. In recent phase 3 trials that used strict response assessment criteria, the response rates with single-agent DTIC did not exceed 12% [5].

Over the past 30 years after its approval by the FDA, DTIC remains the only currently used cytotoxic drug for the treatment of metastatic melanoma. Despite its low single-agent activity, DTIC has remained the mainstay of many combination chemotherapy regimens and evaluations of resistance-reversing agents. After more than 20 years of research, DTIC is still the standard against which most new chemotherapy agents are compared [6].

As for the dose, it has been demonstrated that 850–1000 mg/m<sup>2</sup> single dose of DTIC is tolerated. This single dose administration appears to deliver clinical improvements similar to those observed with multiple doses that provide the same total dose per cycle. This should be the reference standard for randomized trials comparing new therapies with DTIC [7].

## 2.2. Temozolomide

Temozolomide (TMZ) is an orally active alkylating agent. It's a prodrug of MTIC and congener of DTIC. It has been available in the US since August 1999 and in other countries since the early 2000s. The therapeutic benefit of temozolomide depends on its ability to alkylate/methylate DNA, which most often occurs at the N-7 or O-6 positions of guanine residues. This methylation damages the DNA and triggers the death of tumor cells. However, some tumor cells are able to repair this type of DNA damage, and therefore diminish the therapeutic efficacy of temozolomide, by expressing an enzyme called O-6-methylguanine-DNA methyltransferase (MGMT) or O-6-alkylguanine-DNA alkyltransferase [8].

The single agent activity of TMZ in metastatic melanoma has been established in several phase 1 and 2 studies [9]. In a randomized trial of 305 patients with advanced melanoma, TMZ showed efficacy at least equivalent to that of DTIC in terms of objective response rate, time to progression, and overall disease-free survival [10]. TMZ was tolerated very well and showed an advantage in terms of improvement in the quality of life. More patients showed improvement or maintenance of physical functioning at Week 12. That trial excluded patients who had brain metastases. Because the trial design was intended to demonstrate the superiority of TMZ over DTIC, rather than equivalence, the FDA did not accept the results of that trial as grounds for approving a melanoma indication for TMZ. But in clinical practice, patients with metastatic melanoma often are treated off-label with TMZ.

TMZ has demonstrated efficacy in the treatment of variety of solid tumors, especially in brain malignancies, which is a manifestation of its far greater ability to penetrate the central nervous system (CNS). Taking into account the high rate of CNS recurrence as a site of failure after cytotoxic chemotherapy, TMZ may represent a viable alternative to DTIC, which is ineffective against melanoma CNS metastases.

## 2.3. Sorafenib

Sorafenib (BAY43-9006, developed by Bayer Pharmaceuticals, West Haven CT, trade name Nexavar) is an orally administered tyrosine kinase inhibitor. It is a potent inhibitor of the BRAF kinase that is frequently mutated in melanoma, as well as an inhibitor of the Vascular Endothelial Growth Factor (VEGF) receptor and other kinases. It targets the adenosine triphosphate-binding site of the BRAF kinase and inhibits both wild-type and mutant BRAF *in vitro*. Sorafenib was approved by the FDA in December 2005 for use in the treatment of advanced renal cancer. Preclinical studies demonstrated a significant retardation in the growth of human melanoma tumor xenografts with Sorafenib. In a phase 1 study, the maximum tolerated dose of Sorafenib as a single agent was established at 400 mg twice daily, and the

most common toxicities were gastrointestinal (mainly diarrhea), dermatologic (skin rash, hand-foot syndrome), and fatigue [11].

But in further phase 2 clinical trials, Sorafenib had shown relatively little activity in metastatic melanoma when using alone. In a phase 2 trial that was conducted in 20 patients with refractory metastatic melanoma, Sorafenib showed modest activity with 1 partial response and 3 patients who achieved stable disease [12]. In another phase 2, randomized, discontinuation trial, no objective responses were achieved, and 19% of patients achieved stable disease [13].

Sorafenib combined with other chemotherapy drugs were also tested clinically. In a phase 1 and 2 study that combined carboplatin and paclitaxel with escalating doses of Sorafenib in 35 patients, a promising response rate of 31% was observed, and another 54% of patients experienced stable disease that lasted longer than 3 months. That study recently was updated to include 105 patients, and the current response rate is 27% [14]. On this basis, 2 phase 3 trials have been launched to assess the efficacy of carboplatin and paclitaxel plus Sorafenib versus placebo in chemotherapy-naïve patients and in previously treated patients. In December 2006, Bayer reported the combinations failed to show significant improvement of progression-free survival in melanoma patients [15].

## 2.4. Vemurafenib

Vemurafenib established a successful model for extracellular chemotherapeutic targeted therapy based on deep understanding cancer biology. It's a paradigm of structured-based drug development. It was first discovered in 2002 that the protein kinase BRAF is mutated in about 70% of malignant melanomas and a significant number of colorectal, ovarian and papillary thyroid cancers, implicating mutated BRAF as a critical promoter of malignancy. Then scientists determined the structure of the BRAF catalytic domain and identified a class of BRAF inhibitors that bind to the active conformation of the protein. Further lead series were developed and crystal structures of complexes combined with molecular modeling studies have resulted in potent selective inhibitors. Vemurafenib is the first one that went into clinical trials and gained FDA approval in August 2011.

Vemurafenib (PLX4032/RG7204) is developed by Plexxikon (now part of the Daiichi Sankyo group and Hoffmann–La Roche) for the treatment of late-stage melanoma. Vemurafenib can induce programmed cell death in melanoma cell lines. It interrupts the BRAF/MEK step on the BRAF/MEK/ERK pathway – if the BRAF has the common V600E mutation [16].

Vemurafenib has very impressive single-agent clinical activity, with unprecedented response rates of about 80% and a clear impact on progression-free survival longer than 6 months. An international randomized open-label trial in patients with previously untreated metastatic or unresectable melanoma with the BRAF<sup>V600E</sup> mutation led to the FDA approval of Vemurafenib for melanoma. This clinical trial enrolled 675 patients. 337 patients were randomly assigned to vemurafenib with 960 mg orally twice daily. 338 patients were randomly assigned to dacarbazine with 1000 mg/m<sup>2</sup> intravenously every three weeks. Treatment end-points are disease progression, unacceptable toxicity, and/or consent withdrawal.



Vemurafenib's efficacy was measured by overall survival (OS), investigator-assessed progression-free survival (PFS) and confirmed investigator-assessed best overall response rate. Overall survival was significantly improved in patients receiving vemurafenib compared with those receiving dacarbazine. The median survival of patients receiving vemurafenib had not been reached and was 7.9 months for those receiving dacarbazine. Progression-free survival (PFS) was also significantly improved in patients receiving vemurafenib. The median PFS was 5.3 months for patients receiving vemurafenib and 1.6 months for patients receiving dacarbazine. 48.4% for patients who received vemurafenib showed complete or partial response while only 5.5% patients who received dacarbazine showed complete or partial response [17].

Arthralgia, rash, photosensitivity, fatigue, alopecia, pruritis, and skin papilloma were observed in at least 30% of patients treated with vemurafenib. Cutaneous squamous cell carcinomas were detected in approximately 24% of patients. Other adverse reactions reported in patients treated with vemurafenib included hypersensitivity, Stevens-Johnson syndrome, toxic epidermal necrolysis, uveitis, QT prolongation, and liver enzyme laboratory abnormalities [18].

## 2.5. Other single chemotherapy agents

Cisplatin and carboplatin have shown modest activity as single agents in patients with metastatic melanoma. Cisplatin as single-agent therapy induced a 15% response rate with a short median duration of 3 months [19]. A response rate of 19% has been reported in 26 chemotherapy-naïve patients with metastatic melanoma who received carboplatin. In those patients, there were 5 partial responses, and thrombocytopenia was the dose-limiting toxicity [20]. *In vitro* studies suggested that oxaliplatin may be more active than cisplatin or carboplatin. But a small phase 2 trial in 10 patients who had received and failed prior chemotherapy produced no objective responses [21].

The nitrosoureas (carmustine, lomustine, and semustine) induce objective responses in 13~18% patients. They can cross the blood-brain barrier. But at conventional doses, little or no activity was observed against melanoma brain metastases [22]. Another drawback of the nitrosoureas is they induce prolonged myelosuppression. Despite these, they have been included frequently in multi-agent chemotherapy combinations, presumably for their ability to penetrate into the CNS and lack of viable alternatives for metastatic melanoma.

The vinca alkaloids (vindesine and vinblastine) have produced responses in approximately 14% of patients [23]. The taxanes have produced responses in 16~17% patients [24]. All of these response rate data were obtained from phase 2 trials. None of those drugs have been evaluated as single agents in phase 3 trials. Based on the experience with DTIC, it is likely that the phase 3 trial objective response rates would be less than the rates reported from phase 2 trials. All of these drugs are rarely used currently as single-agent therapy in metastatic melanoma, but they frequently have been incorporated into combination chemotherapy and biochemotherapy regimens.

## 2.6. Chemotherapy drug combinations

Theoretically drug combination should be based on laboratory or clinical evidence of synergistic effect. But since single-agent chemotherapy regimens only provided modest activity against metastatic melanoma and lack of viable alternatives, many combination regimens have been evaluated in clinical trials. Initially two-agent combinations were tested in which DTIC was combined with a nitrosourea, vinca alkaloid, or platinum compound. In most of these trials, only 10~20% response rates were observed. There was little evidence to suggest superiority of these combinations compared with DTIC treatment alone [25-27].

In order to improve response rates, more aggressive multi-drug combinations using 3 or 4 different drugs were also tested clinically. Two most widely studied combinations are cisplatin, vinblastine, and DTIC (CVD) and the Dartmouth regimen. The latter is a 4-drug combination consisting of cisplatin, DTIC, carmustine, and tamoxifen (also called CDBT). Both combinations showed improved response rates that ranged from 30% to 50% in single-institution phase 2 studies [28, 29]. But in further randomized phase 3 trials which involved more patients, they all showed much lower efficiency: In a randomized trial comparing CVD with single-agent DTIC that involved approximately 150 patients, the CVD arm produced a 19% response rate compared with 14% for the DTIC arm, and there was no differences in either response duration or survival. In another randomized phase 3 trial, the CDBT combination was compared with single-agent DTIC. That cooperative group trial involved 240 patients, and the response rate was 10% for the DTIC regimen compared with 19% for the CDBT regimen ( $P=0.09$ ). The median survival was 7 months, with no significant difference between the 2 treatment arms [6].

The main reason for such discrepancies between the results from single-institution studies and those from large, multicenter, cooperative trials probably is selection bias. Differences in performance status, percentages of patients with visceral involvement, and number of metastatic sites easily could account for some of the observed differences. In fact, all of those factors are known to have an impact on both response rate and survival [30].

Overall, controlled trials have produced no compelling evidence to support the value of combination chemotherapy, with or without tamoxifen, in patients with metastatic melanoma. Toxicity was substantially greater for the combination regimen, with bone marrow suppression, nausea, emesis, and fatigue significantly more frequent with CDBT than with DTIC [6]. So it is difficult to justify the use of either CVD or CDBT instead of single-agent DTIC or TMZ for the treatment of most patients with metastatic melanoma.

## 3. Immunotherapy agents

### 3.1. Interleukin-s (IL-2)

In 1998, the FDA approved intermittent high-dose bolus IL-2 based on its ability to mediate durable complete response in metastatic melanoma patients [31]. IL-2 is a type of cytokine immune system signaling molecule, which is a leukocytotropic hormone that is instrumen-

tal in the body's natural response to microbial infection and in discriminating between foreign (non-self) and self. It's a glycosylated 15,500 dalton single protein molecule. IL-2 mediates its effects by binding to IL-2 receptors, which are expressed by lymphocytes, the cells that are responsible for immunity. It is one of the only two FDA approved agents for the treatment of metastatic melanoma. Although the overall response rate is only about 15% and less than 5% of patients achieve complete remission with IL-2, its performance on melanoma is better than DTIC.

One of the major immunologic effects of IL-2 upon the immune system is to expand the total number of T-lymphocytes (CD4+ and CD8+) and to prevent lymphocyte apoptosis. Another key role of IL-2 is to provide the appropriate cytokine milieu necessary to overcome tumor-induced immune tolerance. But the exact molecular and genetic mechanisms involved in this complex interaction between the tumor and the host immune response is still largely unknown [32].

There is currently a wide spectrum of dosing schedules and regimens for IL-2 therapy, with the current standard used by most oncologists being 600,000 to 720,000 IU/kg/dose, given at 8 h intervals. Although the optimal dosing schedule resulting in the best clinical response is currently unknown, previous data would suggest that the higher dose regimens as well as the number of total doses received correlates best with clinical response. Thus, several groups have begun to look at alternative dosing strategies to achieve an increased drug tolerance and tolerability profile, such as the continuous infusion of IL-2 (18 mIU/m<sup>2</sup>/day) over an extended period of 72 h [33]. But the multiorgan toxicity of many IL-2 regimens limits its use. In addition, the tumor-killing cytotoxic T cells and natural killer cells, which are the presumed target cells for IL-2, are frequently inefficient in the tumor environment, partly due to suppressive and apoptosis-inducing signals from tumor-infiltrating mononuclear phagocytes [34].

### 3.2. Interferon $\alpha$

Interferons (IFNs) are proteins made and released by the cells of most vertebrates in response to the presence of pathogens or tumor cells. They allow communication between cells to trigger the protective defenses of the immune system that eradicate pathogens or tumors. IFNs belong to the large class of glycoproteins known as cytokines. They are named after their ability to "interfere" with viral replication within host cells. IFNs have other functions: they activate immune cells, such as natural killer cells and macrophages; they increase recognition of infection or tumor cells by up-regulating antigen presentation to T lymphocytes; and they increase the ability of uninfected host cells to resist new infection by virus.

Based on the type of receptors through which they signal, human interferons have been classified into three major types. The type I interferons present in human are IFN- $\alpha$ , IFN- $\beta$  and IFN- $\omega$  [35]. High-dose IFN therapy using IFN- $\alpha$  was the first form of medical therapy to be approved by the FDA for use in high-risk melanoma in the adjuvant setting. Adjuvant normally means using IFN- $\alpha$  weeks after the surgical excision of the melanoma tumor. Common treatment scheme is IFN- $\alpha$ 2b at 20 million units (MU)/m<sup>2</sup>/day intravenous injection 5 days a week for 4 weeks, then 10 MU/m<sup>2</sup>/day subcutaneous injection 3 days a week for the



next 48 weeks for a full year's. But IFN- $\alpha$ 2b can also be used one month before definitive surgical lymphadenectomy. This is called 'neoadjuvant' treatment [36].

The first randomized comparison of high-dose IFN versus observation found the median relapse-free survival was 1.72 years in the high-dose IFN arm versus 0.98 year in the observation arm ( $P=0.0023$ ) and the median overall survival was 3.82 versus 2.78 years ( $P=0.0237$ ) respectively [37]. But in a later pooled analysis of more patients in more clinical trials, the relapse-free survival benefit was maintained but no overall survival benefit was seen [38].

The exact mechanism of IFN IFN- $\alpha$ 's anti-tumor efficacy is still unknown. But it was found that the STAT1/STAT3 expression ratios rose in association with IFN treatment. The clinical effects of IFN- $\alpha$ 2b in human melanoma are also found to be inversely related to STAT3 expression (41). Induction of apoptosis has been shown to be important *in vitro*, if not *in vivo*. IFN- $\alpha$  can induce apoptosis in transformed cell lines as well as primary tumor cells [39].

High-dose IFN is the standard of care for high-risk melanoma patients in the adjuvant setting. However, it is associated with significant toxicity. The incidence and severity of these adverse events is clearly dose-related. Consequently, there has been a great deal of interest in intermediate- and low-dose regimens administered through subcutaneous injection. However, none of the trials using intermediate or low dosing so far have been able to demonstrate any reliable benefit in terms of relapse-free survival or overall survival [40].

### 3.3. Pegylated interferon- $\alpha$ 2b

Pegylated interferon- $\alpha$ 2b gained its approval from the FDA in March 2011 in the adjuvant setting for melanoma patients with lymph-node-positive disease (stage III) after lymph-node dissection. The approval was based on a randomized controlled phase-III trial in 1256 stage-III melanoma patients. This trial compared treatment with pegylated interferon-  $\alpha$ 2b for up to 5 years with observation. The results revealed a significant and sustained impact on relapse free survival (RFS) in the intention-to-treat (ITT) population. This trial also showed that interferon- $\alpha$ 2b treatment didn't significantly improve distant metastasis-free survival (DMFS) or overall survival (OS) [41]. Pegylated interferon- $\alpha$ 2b also showed much better effect in patients with sentinel-node-positive disease (stage III-N1: microscopic involvement only) compared with patients with palpable nodal disease (stage III-N2). It also significantly improved DMFS in sentinel-node-positive patients in contrast to a marginal effect in patients with palpable nodes. The authors identified tumor stage as a predictive factor in trials. One very important finding from clinical trials was that ulceration of the primary melanoma indicated a distinct biology that was clearly IFN sensitive in contrast to the non-ulcerated type of melanoma.

### 3.4. Anti-CTLA4 antibodies: Ipilimumab and tremelimumab

Cytotoxic T-Lymphocyte Antigen 4 (CTLA4) also known as CD152 (Cluster of differentiation 152) is a member of the immunoglobulin super family, which is expressed on the surface of Helper T cells and transmits an inhibitory signal to T cells that eventually shuts off the activated state. The rationale for involving this in treatment of metastatic melanoma is to

block the negative signal sending by CTLA4 by using anti-CTLA4 antibodies, thus reduce the sensitivity of activated T cells to negative regulatory signals and enhance the immune response of the host to tumor cells.

As of October 2007 there are two fully human monoclonal anti-CTLA4 antibodies in advanced clinical trials, one from Medarex, Inc. (Princeton, NJ) and Bristol-Myers Squibb (New York), called ipilimumab (MDX-010), and one from Pfizer (New York), called tremelimumab (formerly ticilimumab, CP-675,206) [42]. These antibodies were produced using different types of mice with engineered immune systems, and are thus fully human, with long half-lives of 2–4 weeks.

Ipilimumab (MDX-010) is an IgG1 monoclonal antibody. Preclinical and early clinical studies of patients with metastatic melanoma show that ipilimumab promotes antitumor activity as monotherapy and in combination with treatments such as chemotherapy, vaccines, or cytokines. The initial success with these antibodies has encouraged the rapid development of new agonistic and antagonistic antibodies that alter immune regulation, such as anti-PD-1, anti-4-1BB, anti-CD40, and anti-OX-40. On December 10, 2007, Bristol-Myers Squibb and Medarex released the results of three studies on ipilimumab [42]. The three studies tested 487 patients with metastatic melanoma. Short-term tumor progression prior to delayed regression has been observed in ipilimumab-treated patients, and objective responses may be of prolonged duration. In some patients clinical improvement manifests as stable disease, which may also extend for months or years. One of the three studies failed to meet its primary goal of shrinking tumors in at least 10% of the study's 155 patients. Overall the medication produced weaker-than-anticipated efficacy on melanoma patients.

In the meantime, the side effect profile was high in the ipilimumab treated group, with the generation of autoimmune-like effects, such as diarrhea, dermatitis and effects upon the thyroid and pituitary glands. Several patients also experienced vitiligo, indicative of anti-melanocyte autoimmunity. However, the majority of the side effects were noted to be transient (except the vitiligo), improving or disappearing after the completion of therapy. Early clinical data suggest a correlation between these side effects and response to ipilimumab treatment and most likely reflect the drug mechanism of action and corresponding effects on the immune system [42].

In 2011, the first-line pivotal trial data for ipilimumab was released. The median survival of patients treated with ipilimumab at a dose of 10 mg/kg in combination with dacarbazine was 11.2 months. Patients treated with dacarbazine alone showed a median survival of 9.1 months. The improvement in the median survival was 2.1 months. The estimated survival rates in the two groups of, respectively, 47.3% and 36.3% at 1 year, 28.5% and 17.9% at 2 years, and 20.8% and 12.2% at 3 years. These results are not better than those observed with the 3 mg/kg dose in second-line treatment. One possible reason is that a significant and unexpectedly high rate of hepatitis in the dacarbazine + ipilimumab arm did take a significant percentage of patients off treatment before the third or especially the fourth dose of ipilimumab could be administered, thus limiting both the number of administrations of dacarbazine as well as of ipilimumab in the combination arm. Other immune-related adverse events were not increased compared to those with the 3 mg/kg dose experience. The overall inter-

pretation therefore is that dacarbazine did not help, but may rather have mitigated the results in the dacarbazine plus ipilimumab arm. Based on all the data available, the large cumulative phase-II experience, and the two phase-III trials, the FDA approved treatment of melanoma with ipilimumab alone at the 3mg/kg dose [43].

Tremelimumab is an IgG2 monoclonal antibody produced by Pfizer. It blocks the binding of the antigen-presenting cell ligands B7.1 and B7.2 to CTLA-4, resulting in inhibition of B7-CTLA-4-mediated down-regulation of T-cell activation. Subsequently, B7.1 or B7.2 may interact with another T-cell surface receptor protein, CD28, resulting in a B7-CD28-mediated T-cell activation unopposed by B7-CTLA-4-mediated inhibition. Tremelimumab is thought to stimulate patients' immune systems to attack their tumors. It has been shown to induce durable tumor responses in patients with metastatic melanoma in phase 1 and phase 2 clinical studies [44].

On April 2, 2008, Pfizer announced that it has discontinued a phase 3 clinical trial for patients with metastatic melanoma after the review of interim data showed that the trial would not demonstrate superiority to standard chemotherapy [45]. Studies for other tumors are planned as of October 2009, namely for prostate cancer and bladder cancer.

### 3.5. Anti-integrin antibody: Etaracizumab

Etaracizumab (also known as etaratuzumab, MEDI-522, trade name Abegrin) is an IgG1 humanized monoclonal antibody directed against the  $\alpha V\beta 3$  integrin.  $\alpha V\beta 3$  is essential for endothelial cell proliferation, maturation, and survival. When it is blocked, proliferating endothelial cells undergo apoptosis and regress. In addition,  $\alpha V\beta 3$  is highly expressed in melanomas and is associated with tumor growth and invasion. In preclinical studies using  $\alpha V\beta 3$  antagonists, inhibition of melanoma tumor growth independent of its antiangiogenic effects was reported [46]. Etaracizumab has been investigated in 3 phase 1, dose-escalation studies in patients with refractory melanoma. In the phase 2 trial, 57 patients received etaracizumab alone, and 55 patients received etaracizumab plus DTIC. Etaracizumab with or without DTIC generally was well tolerated and was active in patients with metastatic melanoma. The median survival was 12.6 months for the group that received etaracizumab with DTIC and 9.4 months for the group that received etaracizumab without DTIC [47]. These results encouraged people to further test this antibody in more clinical trials.

Early 2010, a study by the Etaracizumab Melanoma Study Group was reported. In this study, 112 patients were randomized to receive etaracizumab alone or etaracizumab plus DTIC. None of the patients in the etaracizumab alone study arm and 12.7% of patients in the etaracizumab plus DTIC study arm achieved an objective response. Stable disease occurred in 45.6% of patients in the etaracizumab alone study arm and 40.0% of patients in the etaracizumab plus DTIC study arm. Despite a modest increase in survival, 12.6 months in the etaracizumab alone arm, versus 9.4 months in the etaracizumab plus DTIC arm, the researchers concluded that the survival results in both treatment arms of this study were considered unlikely to result in clinically meaningful improvement over DTIC alone [48]. At the present time, clinical development of etaracizumab has been interrupted.

### 3.6. Vaccines based on tumor cells: Canvaxin, melacine, and MVax

The basic idea is to use tumor cell-based vaccine to stimulate and activate the host immune system to recognize, contain and eliminate cancer cells. This effect may be based on the following two pathways: direct migration of the tumor cells to the draining lymph node basin after injection, or uptake of apoptotic or necrotic tumor cells by host dendritic cells located within the skin [49].

The most extensively studied tumor cell-based vaccine is a polyvalent, antigen-rich whole cell vaccine called Canvaxin (CancerVax Corp., Carlsbad, CA). It is comprised of three melanoma cell lines that contain over 20 immunogenic melanoma tumor antigens, given intradermally every two weeks for 3 to 5 doses, followed by monthly injections for the remainder of the first year. However, several small, single-institution phase 1 and 2 clinical trials of Canvaxin have not yielded a striking clinical benefit in most patients when administered with BCG as an immunoadjuvant [50]. But the rare complete responder to Canvaxin therapy has prompted the initiation of two multicenter phase 3 randomized trials of Canvaxin therapy in 1998. In these trials, patients who have undergone complete resection of regional (stage III) or distant (stage IV) metastatic melanoma receive postoperative adjuvant immunotherapy with Canvaxin plus *Bacillus of Calmette and Guérin* (BCG) or BCG alone. In April 2005, CancerVax announced the discontinuation of their phase 3 clinical trial of Canvaxin in patients with Stage IV melanoma based upon the clinical funding that it was unlikely that the trial would provide significant evidence of a survival benefit for Canvaxin-treated patients versus those receiving placebo. On October 3, 2005, CancerVax announced the discontinuation of another phase 3 clinical trial of Canvaxin in patients with Stage III melanoma base on a similar reason [51].

The second tumor cell-based vaccine that has been well studied since 1988 is Melacine. It is an allogeneic melanoma cell lysate combined with an immunologic adjuvant which is composed of a mixture of detoxified endotoxin, cell wall cytoskeleton and monophosphoryl lipid A. Early phase 1 and 2 clinical trials in 1987 and 1988 revealed some promising results, with one complete and three partial responses seen in 25 patients treated with Melacine. These results prompted the completion of seven open-label phase 2 trials involving 139 patients with stage III/IV melanoma and a multicenter phase 3 clinical trial of Melacine versus the Dartmouth regimen. The objective response rates for all of the above studies have been between 5 and 10%. Based largely upon these former results and the clinical results of other phase 3 trials, a phase 3 observation controlled trial of Melacine in melanoma patients was conducted. But the results revealed no evidence of a benefit from Melacine in patients with melanoma [52].

One very promising autologous cell vaccine is MVax which is now in active phase 3 clinical trial sponsored by AVAX Technologies, Inc. This vaccine is derived from autologous tumor cells that have been irradiated and then modified with the hapten dinitrophenyl (DNP) [53]. In February 2004 the *Journal of Clinical Oncology* published an article by Dr. David Berd on the treatment of 214 Stage IIIB and IIIC melanoma patients that showed a five-year survival rate of 44%. Comparison to published results of similar patients treated with surgery alone showed five-year survival figures of 22%. In stage IV patients MVax has demonstrated sig-



nificant response rates as a monotherapy and in published reports MVax plus adjuvant IL-2 have reported response rate of 35% (13% Complete Response, 22% Partial Response). This compares to published response rates in low dose IL-2 of 3% [54].

In October 2006, AVAX obtained a Special Protocol Assessment (SPA) agreement with the FDA for its phase 3 protocol. The SPA allows for the start of the phase 3 registration clinical trial for MVax for the treatment of patients with metastatic melanoma. In addition, the SPA addressed AVAX's ability to use a surrogate endpoint as a basis for accelerated approval. Based on this SPA, a phase 3 trial for stage IV melanoma was started on May 2007. AVAX plans to enroll up to 387 patients who will be assigned in a double-blind fashion at a 2:1 ratio to MVax or placebo vaccine. The MVax arm will consist of an initial dose of MVax followed by cyclophosphamide and then six weekly doses of MVax administered with BCG. Following vaccine administration patients will receive a specific schedule of low dose IL-2. Patients assigned to the control group will receive a treatment identical to the MVax group, except that a placebo vaccine will replace MVax. The primary endpoints of the study are best overall anti-tumor response rate and the percentage of patients surviving at least 2 years. Secondary endpoints of the study will include overall survival time, response duration, percentage complete and partial responses, progression free survival and treatment related adverse events [55].

### **3.7. Vaccines based on peptides: MDX-1379, astuprotimut-R, and others**

The identification of tumor antigens that are present on the surface of melanoma cells is the basis for developing cancer vaccines that utilize peptide based immunotherapy. There are several melanoma differentiation antigens known involved in the synthesis of melanin and recognized by melanoma-reactive T cells, for example, gp100, MART-1/Melan-A, tyrosinase, TRP-1 and TRP-2, NY-ESO-1 and the melanoma-associated antigen (MAGE) *etc.* One big advantage of peptide based-vaccination is that it has few toxic side effects or adverse reactions. Data suggests that most tumor cell lines established from fine needle aspiration biopsies of patients with metastatic melanoma exhibit a relatively homogeneous co-expression of MART-1 and tyrosinase, with a much more heterogeneous expression of other tumor antigens, such as gp100, NY-ESO 1 and the MAGE antigens [56].

Rosenberg and his colleagues developed a with a peptide based-vaccine using modified immunodominant peptide of the gp100 antigen, g209-2M. They used this agent vaccinated stage IV melanoma patients subcutaneously every three weeks. Following two immunizations, 10 of 11 (91%) of patients showed a consistently high level of immunization against the native g209~217 peptide, but not against the control peptide g280~288. This study also demonstrated that the majority of patients immunized with the g209-2M peptide in incomplete Freund's adjuvant (IFA) consistently developed high levels of circulating immune precursors reactive against the native g209~217 peptide. Clinically, one of nine patients who received the g209~217 peptide in IFA experienced an objective cancer regression that lasted 4 months. Three of the eleven patients exhibited mixed responses with complete or partial regression of several lesions. However, all patients eventually developed progressive disease [57].



MDX-1379 vaccine consists of two gp100 melanoma peptides. These peptides are part of a protein normally found on melanocytes, or pigmented skin cells, and on melanoma cells. These melanoma peptides are recognized by cytotoxic T cells in melanoma patients that are positive for HLA-A2, a human immune system compatibility antigen that is expressed in approximately half of the melanoma population. Phase II data show limited evidence of MDX-1379's clinical activity although there is strong proof-of-concept for therapeutic vaccines based on gp100 in melanoma. Medarex is currently conducting a phase 3 clinical trial with ipilimumab and MDX-1379 combination therapy in stage III and IV melanoma at multiple sites within the United States. Preliminary data showed MDX-1379 plus ipilimumab induced a modest percentage of durable response in stage IV melanoma. But autoimmune events could make the risk/benefit ratio for MDX-1379 plus ipilimumab unfavorable [58].

Astuprostimut-R (also called recombinant MAGE-A3 antigen-specific cancer immunotherapeutic GSK1203486A) is a cancer vaccine consisting of a recombinant form of human melanoma antigen A3 (MAGE-A3) combined with a proprietary adjuvant with potential immunostimulatory and antineoplastic activities. Upon administration, astuprostimut-R may stimulate a cytotoxic T-lymphocyte response against tumor cells expressing the MAGE-A3 antigen, resulting in tumor cell death. MAGE-A3, a tumor-associated antigen (TAA) originally discovered in melanoma cells, is expressed by various tumor types including melanoma, non-small cell lung cancer, head and neck cancer, bladder cancer, with no expression in normal cells. MAGE-A3 protein has been in-licensed by GlaxoSmithKline (GSK) from the Ludwig Institute for Cancer Research. The proprietary immunostimulating adjuvant in this agent is composed of a specific combination of immunostimulating compounds selected to increase the anti-tumor immune response to MAGE-A3. Using this vaccine as intramuscular administration together with GSK's two proprietary adjuvant systems, AS15 or AS02B, they have developed a treatment regimen for cancer patients called Antigen-Specific Cancer Immunotherapeutic (ASCI).

In 2008, GSK reported a randomized, open-label phase 2 study designed to evaluate Astuprostimut-R. A total of 72 patients with measurable metastatic MAGE-A3-positive cutaneous melanoma (unresectable or in transit stage III or stage IV M1a) were randomized to receive immunization with MAGE-A3 protein combined with either AS15 or AS02B as first-line metastatic treatment. Patients were to receive a maximum of 24 immunizations over four years. Clinical activity is assessed by the Response Evaluation Criteria In Solid Tumors (RECIST) criteria, the international standards for evaluation of solid tumors. Complete response (CR) and partial response (PR) *i.e.*, disappearance or significant reduction of tumor, were reported in 4 patients in the AS15 group (3 CR and 1 PR) with two of these ongoing for more than two years; in the AS02B arm, 1 patient showed a partial response which lasted for 6 months. The safety profile was similar in both groups with the majority of reported adverse events being mild or moderate local or systemic reactions [59]. Currently this agent still is under phase 2 clinical development for progressive metastatic cutaneous melanoma.

Because melanoma tumors are heterogeneous in their antigenic profile, it is very difficult to make vaccines that can elicit cytotoxic T-cell responses universally in all the host immune systems. Rosenberg's group analyzed 28 different peptide-based vaccines utilized in stage

IV melanoma patients. A total of 381 patients were treated with 370 patients showing no response, 9 patients showing a partial response and 2 patients with a complete response, for an overall objective response rate of only 2.9%. This suggested the lack of effectiveness with this single peptide based vaccination approach [60].

Next logical step is to make vaccines with multiple peptides to overcome tumor cell antigenic heterogeneity. A recent randomized phase 2 trial was performed in 26 patients with metastatic melanoma, vaccinating with four melanoma peptides. Although a high level of specific T-cell responses were noted (in 42% of the peripheral blood, 80% of sentinel lymph nodes), only three patients had a clinical response [61].

Here is the biggest issue in this area, actually many peptide based-vaccinations have resulted in a significant increase in the number of lymphocyte precursors reactive against a variety of tumor differentiation antigens by immunization with native or modified peptides. However, such immunological responses to peptide-based therapy have not translated into meaningful clinical responses for the vast majority of patients. To date, there is no study that has clearly shown a direct correlation between an immunologic response to therapy (immune cell activation) and a clinical response (regression of established tumor).

### 3.8. Vaccines based on dendritic cells

In the normal human epidermis and dermis, dendritic cells (DC) are present as relatively immature antigen presenting cells, exhibiting relatively low levels of class II major histocompatibility complex (MHC) molecules and co-stimulatory molecules. But these immature DC are quite capable of capturing various soluble protein antigens, such as apoptotic and necrotic tumor cells and then cross-presenting such tumor-associated antigens to cytotoxic CD8<sup>+</sup> T cells. When relatively immature DC in the skin is triggered to enter afferent lymphatic channels, this migrating pathway also initiates a phenotypic conversion that has profound immunological consequences [30]. When the DC arrives in the lymph node, it is characterized by an abundant levels of class II MHC antigens, as well as high surface levels of costimulatory molecules, such as CD40, CD54, CD80, CD83, and CD86. The matured DC is then capable of forming stable MHC class II-peptide complexes available to activate antigen specific CD4<sup>+</sup> T cells [62].

To make the dendritic cell-based vaccine, the monocyte-derived, autologous DC can be pulsed *in vitro* with either whole irradiated, autologous tumor cells or tumor cell lysate. Once the tumor cells are "fed" to the DC *in vitro*, the apoptotic or necrotic cells are then processed and tumor-specific peptide antigens are then transported to the surface in both an MHC class I- and II-restricted fashion. Both immature and mature DC can be administered to patients as vaccine safely with few adverse side effects. The administration of DC via various routes of vaccination (intradermal, intranodal and intravenous) is also feasible. The first published clinical trial of DC vaccination was in 1995 and has since been followed by 98 additional clinical trials describing more than 1,000 DC-based vaccines performed in 15 different countries. Twenty-eight trials focused on patients with various advanced stages of melanoma. The safety profile was again noted to be quite remarkable, however, despite the

treatment of over 1,000 patients with DC-based vaccines, the record of effectiveness have been disappointing [63].

One very successful DC-based trial for patients with advanced, metastatic melanoma was reported by Nestle *et al.* He used plastic adherent monocytes matured with a xenogeneic-based 10% fetal calf serum, subsequently pulsed with either tumor cell lysate or multiple HLA-matched peptides injected intranodally. This trial involved 16 patients who were immunized on an outpatient basis. Overall, 5 of 16 patients experienced an objective response, 2 complete and 3 partial responses. The side effects were noted to be minimal in all cases, with the development of vitiligo in a few patients. One dramatic feature of this treatment was the durability of the clinical responses, with the 2 complete responders remaining free of disease for over 15 months [64].

One phase 3 clinical trial about using DC-based vaccine to treat metastatic melanoma was report by Schadendorf and colleagues recently [65]. The trial was a prospective, randomized trial that analyzed the therapeutic effects of an autologous peptide-pulsed DC-based vaccine in patients with stage IV melanoma compared to standard chemotherapy with DTIC alone. The results revealed that the overall response in the vaccine group was 3.8% compared to 5.5% in the DTIC group, with no statistically significant differences noted in response, toxicity, overall and progression-free survival between the two groups. The median time to progression was 2.8 months versus 3.2 months respectively and the median survival was 11 months for the DTIC arm but only 9 months for the vaccine arm [65].

Although disappointed by many trials, several new avenues of DC-based immunotherapy are actively being pursued and in various stages of development, focusing on different ways to enhance the therapeutic efficacy of DC in combination with various immunoadjuvants and other anticancer agents.

### **3.9. Individual therapy based on activated T-cells**

One very promising approach to treat metastatic melanoma is to use fully activated anti-tumor T-cells as warhead. This regimen involves the adoptive autologous transfer of highly selective tumor-reactive T-cells directed against over-expressed self-derived differentiation antigens after lymphodepleting chemotherapy. Rosenberg group reported in 2004 a clinical trial using this method. Cancer regression in patients with refractory metastatic melanoma with large, vascularized tumors was noted in a remarkable 18 of 35 patients (51% response rate), including four patients with a complete regression of all metastatic disease. Such results may stem from the ability to infuse a large number of fully activated tumor infiltrating lymphocytes with anti-tumor activity into a host that is depleted of regulatory T-cells [66].

## **4. Gene therapy agents**

The recent developments in the field of gene transfer have advanced the use of gene therapy as a novel strategy against a variety of human malignancies. Because of its unique set of

characteristics, melanoma represents a suitable target for gene therapy. Several strategies have been used by gene therapy to treat melanoma. First is to target melanoma cells to introduce "suicide" genes. Second is to transfer tumor suppressor genes. Third is to inactivate aberrant oncogene expression. Fourth is to introduce genes encoding immunologically relevant molecules. Last is to target the host's immune cells to redirect immune responses against melanoma. Clinical trials have shown the feasibility and safety of gene therapy against malignant melanoma. Although no major successes have been reported, the positive results observed in some patients support the potential for gene therapy in the management of this disease. To make gene therapy as an effective modality of treatment for malignant melanoma, better vector technology as well as increased understanding of the "bystander effect" triggered by gene transfer approaches are needed [67].

The gene therapy in our discussion is to introduce oligonucleotide or DNA sequence into host body thus to stimulate immune response to tumor cells. So it is also called DNA vaccination. This approach has been shown to induce long-lasting immunity against infectious agents and protection from tumor outgrowth in several animal models [68]. Likewise, intramuscular injections of DNA (composed of naked DNA expression plasmids) into humans have also resulted in the development of an immunologic response [69]. It is hypothesized that one mechanism of tumor antigen expression may involve the DNA introducing the appropriate genes into dendritic cells for subsequent processing and presentation to the host immune system. One of the obvious advantages of DNA vaccinations is that they can be administered to patients regardless of HLA-phenotype and without identifying immunogenic epitopes.

#### 4.1. Anti-BCL2 antisense oligonucleotide genasense

Genasense (Oblimersan sodium developed by Genta Inc. which is a biopharmaceutical company based in Berkeley Heights, New Jersey) is a phosphorothioate antisense oligonucleotide directed against the first six codons of the Bcl-2 messenger RNA. Binding of the drug to the mRNA recruits RNase H, resulting in cleavage of the mRNA. As a result, further translation is halted and intracellular protein concentrations of Bcl-2 decrease with time. Melanoma cell lines having Bcl-2 overexpression have been shown to enhance activity of metastasis-related proteinases, *in vitro* cell invasion, and *in vivo* tumor growth [70]. Many *in vitro* studies have demonstrated increased sensitivity of melanoma cells to chemotherapy when combined with antisense Bcl-2 therapy [71]. Genasense is the first oncology drug of its kind to directly target the biochemical pathway (known as apoptosis) whereby cancer cells are ultimately killed by chemotherapy. Genasense is believed to inhibit the production of Bcl-2, a protein that is believed to be a fundamental cause of resistance to anticancer therapy. By inhibiting Bcl-2, Genasense may greatly improve the activity of anticancer therapy.

Encouraged by previous data, numerous clinical trials were started to evaluate the addition of oblimersan to chemotherapy in various solid tumors, including melanoma. Updated analysis from a randomized phase 3 trial, comparing DTIC combined with oblimersan, with DTIC alone in 771 patients with Stage IV or unresectable Stage III melanoma who had not previously received chemotherapy has shown a response rate of 12.4% in the former com-



pared with 6.8% in the latter group ( $P=0.007$ ) [72]. Median progression-free survival for the oblimersan group was 2.4 months as compared with 1.6 months for the DTIC group, with a relative risk reduction of 27% ( $P=0.0003$ ). The median survival was increased from 7.8 months in the DTIC arm to 9 months in the oblimersan arm with a  $P$  value of 0.077, which became significant when the patients with normal baseline LDH were analyzed. In terms of toxicity, no new or unexpected adverse events were observed in this study, which had not been seen with DTIC alone.

However, in May 2004, a new drug application (NDA) based on 6-months of minimum follow-up data from this trial failed to receive an affirmative vote for approval by an advisory committee to the FDA. Genta subsequently withdrew that application, and the Company has not yet made a decision regarding re-filing the U.S. application [73].

#### **4.2. DNA Plasmid-lipid complex allovectin-7**

Allovectin-7 is a bicistronic plasmid formulated with a cationic lipid system containing the DNA sequences encoding HLA-B7 and beta-2 microglobulin, which together form a MHC1 antigen. Injection of Allovectin-7 directly into tumors is designed to stimulate an immune response against both local and distant metastatic tumors. Allovectin-7 is a novel gene therapy approach for cancer with a unique mechanism of action that is fundamentally different from currently approved treatments. The following three mechanisms were believed to play roles in this agent's efficacy. Mechanism one, in HLA-B7 negative patients, a vigorous allogeneic immune response may be initiated against the foreign MHC class I antigen. Mechanism two, in all patients,  $\beta 2$  microglobulin may reconstitute normal class I antigen presentation and/or increase tumor antigen presentation to the immune system. Mechanism three, in some patients, an innate pro-inflammatory response may occur that induces tumor responses following intralesional injection of the DNA/lipid complex. The final outcome of all these mechanisms is to initially cause recognition of the tumor at the local site to allow a then sensitized immune response to recognize un-injected tumors at distant metastatic sites [74].

In 2001, Dr. Richards and his colleagues began a high-dose, 2 mg, phase 2 trial evaluating the Allovectin-7 immunotherapeutic alone for patients with stage III or stage IV melanoma, who have few other treatment options. The high-dose phase 2 trial completed enrollment in 2003. The data showed that the trial had a total of 15 responders among the 127 patients receiving the high dose (11.8%), with four of the patients having complete responses and 11 having partial responses. The Kaplan-Meier estimated median duration of response was 13.8 months. The Kaplan-Meier median survival was 18 months. The safety profile was excellent with no reported Grade 3 or Grade 4 adverse events associated with Allovectin-7 [75].

Allovectin-7 has been granted orphan drug designation for the treatment of invasive and metastatic melanoma by the FDA's Office of Orphan Products Development. Orphan drug designation provides U.S. marketing exclusivity for seven years if marketing approval is received from the FDA



Vical is conducting the AIMM (Allovectin-7 Immunotherapeutic for Metastatic Melanoma) trial, a phase 3 pivotal trial of Allovectin-7 as first-line therapy in approximately 375 patients with Stage III or IV recurrent metastatic melanoma in accordance with a SPA agreement completed with the FDA. The trial is being conducted at approximately 60 clinical sites worldwide. They designed the trial to include patients most likely to benefit from our treatment, and specifically excluded patients with brain or liver metastases, patients previously treated with chemotherapy, and patients with elevated lactate dehydrogenase (LDH) levels.

In January 2010 Vical announced that the company has completed enrollment of the planned 375 subjects in its multinational phase 3 trial of Allovectin-7 in patients with metastatic melanoma. Allovectin-7®'s safety profile is excellent with no drug-related serious adverse events reported to date in the phase 3 trial [74].

### 4.3. Herpes simplex virus based oncoVEX

OncoVEX (GM-CSF) is an enhanced potency, immuneenhanced oncolytic herpes simplex virus type 1 (HSV-1). It is deleted for infected-cell protein gene 34.5 (ICP34.5), providing tumor selective replication, and ICP47 gene which otherwise blocks antigen presentation. In addition, ICP47 deletion increases unique short region protein 11 (US11) gene expression thereby enhancing virus growth and replication in tumor cells. The coding sequence for human granulocyte-macrophage colony-stimulating factor (GM-CSF) is inserted, replacing ICP34.5, to enhance the immune response to tumor antigens released following virus replication.

OncoVEX is developed by BioVex (Woburn, MA). It is a first-in-class oncolytic, or cancer destroying virus, that works by replicating and spreading within solid tumors (leaving healthy cells unaffected), thereby causing cancer cell death and stimulating the immune system to destroy un-injected metastatic deposits. Both modes of action have been clearly validated in the clinic, where multiple patients with metastatic disease progressing at enrollment have been declared disease free.

BioVex recently concluded a 50-patient phase 2 trial for OncoVEX (GM-CSF) as a stand-alone therapy in patients with Stage IIIC and Stage IV melanoma. The trial was designed to measure overall objective response, which is defined as a complete response, where disease is completely eliminated, or partial response, where there is a >50% reduction in disease burden. 74% of patients who entered the study were progressing after having failed prior therapy. 13 objective systemic responses (26% objective response rate) were achieved including eight CRs, seven of which remain free of disease. 12 responses have so far continued for more than 6 months (ranging from 6 to more than 29 months). Responses were observed in patients with all stages of disease, including the complete resolution of un-injected visceral deposits. Adverse effects were primarily limited to transient flu-like symptoms [76].

In April 2009, BioVex Inc. announced that its OPTiM (OncoVEX Pivotal Trial in Melanoma) phase 3 study with OncoVEX (GM-CSF) in previously treated patients with Stage III and Stage IV melanoma had initiated. The study has commenced recruiting patients in the U.S. and with sites in the United Kingdom, Germany and Australia. The OPTiM trial is a multi-

national, open label, randomized study designed to assess the efficacy and safety of treatment with OncoVEX (GM-CSF) as compared to subcutaneously administered GM-CSF in patients with unresectable stage III (b-c) and stage IV (M1a-c) disease. Patients will have received at least one prior therapy for active disease which includes any type of therapy including investigational drugs. A total of 360 patients will be enrolled (240 to the OncoVEX (GM-CSF) arm and 120 to the control arm). The study design was agreed with the FDA under the special protocol assessment process [77].

## 5. Possible reasons for extremely high resistance of metastatic melanoma

Despite an epic number of clinical trials to test a wide variety of anticancer strategies, the average survival rate for patients with metastatic melanoma remains unimproved during the past 30 years (41). Though constant clinical trials effort, although some approaches showed promising intermediate results, still no agent has been granted FDA approval for the treatment of metastatic melanoma. There are several reasons that may account for the extremely high resistance of metastatic melanoma to current treatment modalities.

### 5.1. Reasons for chemotherapy resistance

Melanoma cells are quite resistant to most chemotherapy reagents. This is associated with the specific feature of melanoma cells. In nature, these cells have low levels of spontaneous apoptosis *in vivo* compared with other tumor cell types, and they are relatively resistant to drug-induced apoptosis *in vitro* [78]. The natural role of melanocytes is to protect inner organs from UV light, a potent DNA damaging agent. Therefore, it is not surprising that melanoma cells may have special DNA damage repair systems and enhanced survival properties [79]. Moreover, recent studies showed that, during melanoma progression, it acquired complex genetic alterations that led to hyperactivation of efflux pumps, detoxification enzymes, and a multifactorial alteration of survival and apoptotic pathways. All these have been proposed to mediate the multi-drug resistant phenotype of melanoma [80].

### 5.2. Barriers for successful immunotherapy

The major barrier is immunosuppressive effects activated by tumors. Tumor cell can escape immune rejection and induce immunosuppression through the following five major paths. Firstly, tumor cells may lose or down-regulate either the melanoma associated antigens or MHC molecules. Secondly, tumor cells may produce a plethora of immunosuppressive factors such as interleukin-10, VEGF and transforming growth factor. These factors create an inherently unfavorable microenvironment that limits the host immune response, in addition to tolerating the T-cell response to established tumor. Third possible reason is intrinsic inefficiency of DC whereby the appropriate co-stimulatory molecules are not being presented on the cell surface. Fourth possible reason is tumor-related alterations in T-cell signaling and a skewing of the immune response from a Th1 (immunoactivating) to a Th2 response (immunotolerant). Lastly, the concept of tumor cell escape and immune tolerance is an exceed-

ingly complex process. We need to further understand these mechanisms before we can have successful immunotherapy to melanoma [32].

Specifically for cancer vaccines, there are some further barriers. First is the characterization of vaccines potency and toxicity. This is especially important in the transition from phase 2 to phase 3 trials. To select a meaningful and validated end point for trials is a big challenge most of the time. Second barrier is selection of the maximum tolerated dose of cancer vaccine, particularly compared with traditional anticancer agents. Cancer vaccines are typically not very toxic. So the optimum dose often has to be based on the immune response of patients. But if the patients have previously been heavily treated with other anticancer agents, this can lead to a compromised immune system that makes it difficult to detect an evoked immune response. The third barrier is appropriate trial design and statistical data process. This is also a key part and can substantially affect final trial outcome [53].

## 6. Future directions

With the rapidly rising incidence and the high resistance to current therapeutic agents, developing more effective drugs for metastatic melanoma is urgently needed. But before we can thoroughly understand all the major molecular pathological changes associated with melanoma malignancy, it is very difficult to reach a cure for it.

Melanoma is an extremely complicated disease, with many gene mutation and signaling pathway changes. Elevated signaling pathway in melanoma including mitogen-activated protein kinase (MAPK) pathway, phosphatidylinositol 3 kinase (PI3K)-AKT pathway, Wnt-Frizzled- $\beta$ -catenin pathway, JAK/Stat pathway and  $\alpha$ -MSH-MC1R or microphthalmia-associated transcription factor (MITF) pathway. The first two are crucial pathway accounting for melanoma malignancy. Gene mutation that are involved in melanoma include the following oncogenes: BRAF, N-ras, akt3; tumor suppressors: CDKN2A, PTEN, p53, APAF-1, p16, p15, p19; others: Cyclin D1, MITF etc [81].

The binding of growth factors to their respective receptors leads to activation of RAS proteins. Ras will then activate Raf. Raf activate mitogen-activated protein kinase (MEK), which then act on extracellular-related kinase (ERK). Phosphorylated ERK kinases (ERK-P) translocate to the nucleus and activate transcription factors, which promote cell cycle progression and proliferation. The PI3K-AKT pathway mediates cell survival signaling via growth factors. Phosphatase and tensin homolog (PTEN) inhibits growth factor signaling by inactivating phosphatidylinositol triphosphate (PIP3) generated by PI3K. Activated PI3K converts the plasma membrane lipid phosphatidylinositol 4,5-bisphosphonate to PIP3, which acts as a second messenger leading to the phosphorylation AKT and subsequent up-regulation of cell cycle, growth, and survival proteins. AKT can also up-regulate mTOR (mammalian target of rapamycin), S6K, and NF $\kappa$ b leading to cell growth and inhibition of apoptosis.

Knowing the huge complexity of melanoma, it's easy to understand why so many random trials of single agents or combinational treatment have failed. So targeted therapy in a sys-

temic way based on the understanding about melanoma molecular pathology seems to be a reasonable way to fight this disease.

Individualized T-cell-based therapy is a very promising approach. Combined with other suitable tumor killing agents, it could improve the patient survival rate and time. Unfortunately, the selective tumor-reactive T-cells isolated from a patient can only be used for this same patient. Thus the cost associated with this treatment method is very high. Such an expensive treatment may not be available to all the patients in the near future.

In learning from our efforts in the past, we must continue to challenge the current paradigms of treatment as we forge new paths to more effective treatment options. This will likely involve a multimodal approach to therapy utilizing all of the available tools in our arsenal. Several agents given in unique combinations may then synergize with standard chemotherapeutic regimens resulting in prolonged clinical responses and long term survival. Take Sorafenib as an example, its failure maybe largely due to the fact that it only blocks the RAF-MEK-ERK signaling pathway. Melanoma cells can still survive by compensatory up-regulation in other survival pathways such as the PI3K-AKT pathway. Melanoma can also develop drug resistance with time by over-expressing MDR genes. Ideally, if we can use drugs to synergistically block all the major survival pathways in melanoma cells and then educate our immune system to fight the tumor cells, we will have a much better chance to conquer this deadly disease.

## Author details

Zhao Wang, Wei Li\* and Duane D. Miller

\*Address all correspondence to: wli@uthsc.edu

Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, Memphis, USA

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