

# Expert Opinion

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## Immunotherapy of melanoma: a critical review of current concepts and future strategies

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Advanced melanoma is a devastating disease with a very poor overall prognosis. There are only two agents that are approved by the FDA for use in patients with metastatic melanoma: dacarbazine and IL-2. Both agents have an overall response rate well below 20%, with only rare long-term responders noted. Metastatic melanoma is known to be one of the most resistant cancers to a plethora of treatment modalities, such as single-agent and combination chemotherapy, chemoimmunotherapy and immunotherapy with a host of immune stimulators. Indeed, researchers worldwide have recognized the lack of effective therapies and have refocused their efforts on developing novel and cutting-edge strategies of treatment. This is based on an improved understanding of the complex interactions that occur within the tumor microenvironment, and the central role that the host immune system plays in the surveillance of cancer. This review summarizes the recent results of novel immunotherapeutic regimens and focuses on cutting-edge modalities of treatment that encompass new lines of thinking in the war against cancer and, in particular, melanoma.

**Keywords:** immunoadjuvant, immunotherapy, melanoma, targeted therapy, vaccines

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### 1. Introduction

It should first be clearly stated that the early detection and intervention of cutaneous melanoma will have the most impact on patients with melanoma. For example, when a thin melanoma is recognized early in its course and treated with the appropriate surgical management, > 97% of all patients are cured [1]. For stage III and IV disease, the existing treatment options remain marginal at best, with long-term survival in metastatic melanoma being a relatively rare event [2-4]. This is most clearly highlighted in a recent review by Rosenberg *et al.* [5] who examined the role of immunotherapy in its many forms in the treatment of patients with stage IV melanoma over a 9-year period. They found that the overall objective response rate, using conventional oncologic criteria for clinical tumor response, was only 2.6%. Indeed, even by combining the clinical outcomes of patients with metastatic melanoma worldwide who have participated in an immunotherapy trial (35 trials and 765 patients) there is little improvement in overall response rates, increasing the response to only 3.3%.

Despite intense research efforts, scientists are still struggling with the simplest principles of tumor cell proliferation, cell cycle kinetics and the genetic basis of malignant transformation and tumor progression. Although shining successes are exceedingly rare in this field, efforts must be refocused on those agents that may not provide a cure for metastatic melanoma, but rather provide a situation of stable, non-progressive disease, allowing further understanding of the cellular and molecular

mechanisms of the agents being used. There are several promising compounds and immunotherapeutic approaches that are being used in clinical trials worldwide, providing renewed optimism for patients with advanced melanoma.

### 2. IFN- $\alpha$ and IL-2

IFN is one of the most intensely studied immunotherapeutic agents to date, and the cellular and molecular targets for its action are not yet fully understood. Several trials have been previously conducted with regard to the therapeutic efficacy of IFN- $\alpha$ , with the three largest studies conducted through the Eastern Cooperative Oncology Group (ECOG) under the leadership of J Kirkwood [6-9]. The initial trial, E1684, randomized 280 patients to either IFN 1800 million units/m<sup>2</sup> over 1 year (referred to as high-dose IFN) or to observation [7]. Median overall survival was significantly prolonged at 5 years from 2.8 to 3.8 years ( $p = 0.023$ ) and relapse-free survival was also significantly prolonged by  $\sim 1$  year. The next trial, E1690, compared high- and low-dose IFN- $\alpha$  and observation in a similar high-risk group of adjuvant patients [8]. There was no evidence of an overall survival benefit in any of the arms of this trial, however, it was noted that patients relapsing on the observation arm were salvaged with high dose IFN in a significant proportion of cases, possibly weakening the overall conclusions.

The last ECOG trial (E1694) compared IFN- $\alpha$  with a ganglioside epitope, GM-2, which had shown promising results in Phase I and II clinical trials when compared with a control IFN arm [9]. This trial was terminated early as the interim analysis revealed that there was an increased risk of death in those patients receiving the GM-2 epitope, with a 27% risk reduction in survival for those treated with IFN- $\alpha$ . Other trials have validated the IFN- $\alpha$  experience, with several failing to show clear and conclusive evidence of a significant prolongation of overall survival. A recent meta-analysis has statistically analysed most of the Phase III IFN- $\alpha$  trials worldwide, concluding that treatment with IFN- $\alpha$  reduces the incidence of melanoma recurrence by  $\sim 26\%$  and provides a non-statistically significant benefit in overall survival (15% reduction in risk of death,  $p = 0.06$ ) [10]. The small benefit of IFN- $\alpha$  must be weighed against the long list of adverse side effects and overall cost of the medication, often not covered by many insurance plans within the US and thorough discussion about the risks and benefits of IFN- $\alpha$  therapy should be undertaken with all patients with stage III melanoma, and possibly those with stage II C (T4a, T4b primary tumor) disease due to their poor long-term survival compared with stage III patients.

It is truly an unforgettable experience to witness a patient with metastatic melanoma undergo a complete response to immunotherapy with complete regression of all metastatic disease. This has occurred with IL-2 therapy, originally found to have clinical efficacy in patients with advanced melanoma

and renal cell carcinoma. Since the early studies, IL-2 has played an important role in treating such patients, either alone or in combination with other chemo/immunotherapeutic agents and treatment strategies. IL-2 is the only FDA-approved immunotherapeutic agent used for patients with metastatic melanoma, associated with a low, but consistent, rate of overall response of  $\sim 13 - 17\%$  (7 - 9% partial response and 6 - 8% complete response) [11-13].

There is a wide spectrum of dosing schedules and regimens for IL-2 therapy, with the standard used by most oncologists being 600,000 - 720,000 IU/kg/dose, given at 8-h intervals for 14 planned doses repeated twice over an 8-week period. Although the optimal dosing schedule resulting in the best clinical response is unknown at present, recent data would suggest that the initial clinical response to IL-2 therapy is an independent predictor of improved outcome associated with disease-free survival and the higher dose of IL-2 (720,000 IU/kg) [14]. Some groups have begun to look at alternative dosing strategies in an attempt to increase the overall drug tolerance of IL-2, such as the continuous infusion of IL-2 (18 mIU/m<sup>2</sup>/day) over an extended period of 72 h [15]. Although there are no long-term clinical results yet available, this infusion regimen seems to allow for more IL-2 doses to be administered in a sequential fashion with improved tolerability. Lower doses of IL-2 do not seem to have meaningful clinical efficacy, however, they may have undetermined significance at the immunological level through several hypothetical mechanisms. Thus, the use and efficacy of high-dose IL-2 is well-established for patients with metastatic melanoma, continuing to provide a consistent, albeit low, overall response rate. The most perplexing issue to arise is the inability to identify which patients will respond to IL-2 and which ones will not. Some groups have begun to examine this question, recently analyzing the molecular and genetic changes associated with IL-2 treatment [16,17].

### 3. Chemoimmunotherapy

Chemoimmunotherapy has been evaluated in multiple clinical trials for the treatment of patients with metastatic melanoma. Conventional chemoimmunotherapy can be categorized into two major groups, with the first group involving the use of cytokines combined with cisplatin-based chemotherapy. Several early-phase trials have shown an overall response rate of  $\sim 50\%$  [18-20]. Although this seems surprisingly high, it should be noted that such elevated response rates are of very short duration and often complicated by a high degree of systemic toxicity and adverse side effects. To date, there have been no studies using chemoimmunotherapy that has shown a significant improvement in overall survival [21-24]. Recently, Punt *et al.* [25] performed a randomized, Phase II study of patients with metastatic melanoma: one group received chemoimmunotherapy with dacarbazine, cisplatin, IFN- $\alpha$  and IL-2; the second group received an initial treatment with

two cycles of dacarbazine followed by the same four drugs. They found no significant difference in the median overall survival between the two groups and, therefore, could not recommend two cycles of dacarbazine monotherapy initially in an attempt to select patients for more intense chemoimmunotherapy.

The second strategy is a recent immunotherapeutic approach that has been termed 'immunocentric chemoimmunotherapy'. This approach places the main focus of treatment on immunotherapy, with chemotherapy playing a secondary role, primarily as a modulator of the host immune system. Others have developed a regimen partly comprised of low-dose cyclophosphamide, due to its known function in depleting the regulatory T cell (Treg) population (CD4<sup>+</sup>/CD25<sup>+</sup>) [26-33]. At present, studies are underway to investigate the role of cyclophosphamide combined with other agents that may have a synergistic effect when given concomitantly or in sequential fashion.

The authors have developed a third treatment strategy, termed 'chemocentric chemoimmunotherapy'. In this model, chemotherapy is used as the primary treatment modality with immunotherapeutic strategies to sensitize tumor cells to the cytotoxic effect of the active chemotherapy regimen. This strategy employs the use of activated lymphocytes to presensitize the tumor cells, followed by the administration of chemotherapeutic drugs that are clinically active against the particular tumor type. The rationale for such an approach is based on evidence that activated lymphocytes are known to secrete multiple cytokines that have the ability to regulate tumor cell proliferation and apoptosis. In addition, the activated lymphocytes may exert their direct effect on tumor cells through apoptotic pathways, such as the engagement of Fas-ligand on activated T cells to the Fas receptors present on tumor cells.

This model is potentially applicable to a wide array of tumor histologies and virtually all cancer patients, regardless of their HLA status, because T cells are activated nonspecifically. Preclinical studies examining the role of chemocentric chemoimmunotherapy in melanoma, breast, colon and prostate cancer cell lines have shown a dramatic enhancement of induced tumor-cell apoptosis. This novel strategy may also expand the repertoire of chemotherapeutics that can be used effectively to treat melanoma and other cancers. For example, carboplatin monotherapy has minimal activity against melanoma, breast, colon and prostate cancer; however, using this approach, the viability of tumor cells was reduced by almost 100% with only a single treatment using a suboptimal drug concentration [34].

#### 4. Tumor cell-based vaccines

Several early-phase clinical trials have shown that both autologous and allogeneic tumor cell-based vaccines can be given safely with few adverse side effects. One of the first tumor cell-based vaccines to be extensively tested in clinical trials was a polyvalent, whole-cell vaccine derived from three

melanoma cell lines called onmelatucel-L (CancerVax Corp., Carlsbad, CA, USA). Although early trials failed to show significant clinical impact, there were a few complete and durable responses that provided the impetus for the initiation of two multi-center Phase III randomized trials of this whole-cell vaccine therapy beginning in 1998 [35]. The design of the trial for stage IV patients required that all patients receive definitive surgical removal of all metastatic disease prior to entry into the trial. Unfortunately, both trials were closed prematurely to further accrual due to an interim analysis that revealed no probable efficacy over placebo [35]. Other evidence would support this hypothesis of first removing the bulk of disease, with long-term responders noted in patients who have undergone complete surgical resection of regional nodal disease without any form of adjuvant immunotherapy [36,37].

There have been several other tumor-cell-based trials over the years [38-51] that have yielded a few interesting clinical outcomes; overall, when strict response criteria is employed, the responses rates remain exceedingly low. The next generation of whole tumor cell vaccines has incorporated advances in gene transfer technology with the immunobiology of the cancer cell and host immune system. Initial preclinical studies have shown that a whole melanoma tumor cell vaccine transduced with the granulocyte-macrophage colony-stimulating factor gene conferred a potent and long-lasting antitumor immune response [52]. The secretion of granulocyte-macrophage colony-stimulating factor by the transduced melanoma cells resulted in the attraction of immune cells, such as antigen-presenting cells (APCs) and T lymphocytes, to the vaccine site [52,53]. Based on this and other data, several Phase I/II clinical trials have been completed using this approach, with a few objective clinical responses noted [54-56]. However, these early studies only involve a small numbers of patients and further clinical trials with a larger number of patients will be necessary until stronger conclusions can be made as to the efficacy of this vaccine approach.

#### 5. Other vaccination strategies: peptide and dendritic cell-based vaccines

One of the first clinical trials to use peptides derived from melanoma cells was performed by Rosenberg *et al.* [57], who vaccinated stage IV melanoma patients with a modified immunodominant peptide of the gp100 antigen, g209-2M. They found that 10 out 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. Although many developed an immunologic response to the peptide vaccine, all patients went on to develop progressive disease. However, this study provided a central proof-of-principle that patients with advanced melanoma were readily able to mount an immune response against their own self-antigens, a concept not previously appreciated. Although exciting, it also highlighted that an immunologic response does not equal a clinical response.

When designing a peptide-based approach, it is important to recognize that most tumor nodules are comprised of a heterogeneous population of tumor cells with different levels of tumor antigen expression. It has previously been shown that synchronous lesions of patients with metastatic melanoma are heterogeneous in their tumor antigen expression [58], with data suggesting that daughter cell lines derived from patients with metastatic melanoma exhibit an overall heterogeneous expression of the common tumor antigens, such as gp100, NY-ESO 1 and the melanoma antigen gene antigens [59]. Although seemingly effective at enhancing a tumor antigen-specific immune response, the clinical outcomes have been uniformly disappointing. A recent analysis of 28 different peptide-based vaccines in stage IV patients revealed an overall response rate of only 2.9% [5]. Other studies using a peptide-based vaccination strategy have shown some limited responses to therapy [60,61].

Another novel approach to the immunotherapy of melanoma involves the use of autologous dendritic cells (DCs) as potent APCs with the ability to interact with other immune cells, resulting in the activation of an antigen-specific immune response. The first published clinical trial of DC vaccination was in 1995 and has since been followed by 98 additional clinical trials describing > 1000 DC-based vaccines performed in 15 different countries [62]. A total of 28 trials focused on patients with various advanced stages of melanoma. The safety profile was again noted to be remarkable, however, despite the treatment of > 1000 patients with DC-based vaccines, the record of effectiveness has been disappointing, with overall response rates of < 5% in most cases. A recent DC-based vaccination trial was completed by Schadendorf *et al.* from the German Dendritic Cell Study Group [63]. The trial was a prospective, randomized Phase III clinical trial that analyzed the therapeutic effects of an autologous peptide-pulsed DC-based vaccine in patients with stage IV melanoma compared with standard chemotherapy with dacarbazine alone. The results revealed an overall response rate of 3.8% in the vaccine group compared with 5.5% in the dacarbazine group, with no significant differences noted in median time to progression, overall and progression-free survival. Another recent DC-based vaccination trial has yielded similar results to past DC trials, with an objective clinical response noted in 2 out of 20 patients (1 partial and 1 complete response) [64-67].

### 6. Immunoadjuvants in vaccine therapy

Therefore, due to the poor immunogenicity of tumor vaccines, the help of an immunoadjuvant that has the ability to further stimulate both arms of the immune system may be required.

Several adjuvants have been used in human trials, such as incomplete Freund's adjuvant, Bacillus Calmette-Guerin and keyhole limpet hemocyanin, in an attempt to enhance the immunogenicity of such a vaccine. One such novel immunoadjuvant is the synthetic deoxycytidyl-deoxyguanosine

oligodeoxynucleotides (CpG-ODNs), known to strongly activate innate and adaptive immunity via the selective recognition and binding to Toll-like receptor (TLR)-9. The TLR-9 is primarily expressed in human B cells and plasmacytoid DCs, providing a powerful danger signal with resultant activation of DC, macrophages, monocytes, neutrophils and T cells. A wide range of DNA backbones, modifications and substitutions have been explored for their effects on the capacity of CpG-ODN to activate TLR-9 and induce innate and adaptive immunity. The recently described CpG 7909 has been optimized to stimulate human plasmacytoid DCs and B cells, and has been examined in several preclinical and early phase clinical trials [68-76]. On the contrary, a recent study suggests that human plasmacytoid DC, activated by CpG induces the generation of CD4<sup>+</sup>/CD25<sup>+</sup> Tregs, known to play an important role in the maintenance of immunologic tolerance and immunosuppressive function [77]. Thus, such conflicting molecular actions highlight the necessity to further research such agents and determine their role in future immunotherapeutic trials.

The first human trial of CpG-ODN combined with a T cell peptide antigen was performed by Speiser *et al.* [71] and demonstrated that CpG 7909 is an efficient immunoadjuvant capable of promoting a rapid antigen-specific CD8<sup>+</sup> T cell response. However, this was a Phase I study not designed to assess tumor response and, therefore due to the small number of patients (n = 8) and short follow-up time, conclusions as to the efficacy of this approach cannot be assessed. The clinical use of CpG as either a monotherapy or as an immunoadjuvant combined with a multi-modal vaccine approach in cancer treatment is being assessed in early Phase I and II clinical trials.

Imiquimod is an imidazoquinolone compound that has also been found to have immunomodulatory activities, it was recently approved by the FDA for the topical treatment of actinic keratoses, anogenital warts and superficial basal cell carcinoma. The mechanism of action as a topical immunomodulator is relatively unknown, but data would suggest that imiquimod is able to stimulate the innate immune response through the TLR-7 and other undefined mechanisms linked to cell-mediated immunity. Recently, several groups have reported their clinical results of treatment in patients with primary cutaneous (melanoma *in situ* and lentigo maligna) and metastatic melanoma using 5% imiquimod cream [78-82]. Treatment schedules and regimens were variable, with most trials adjusting the frequency and amount based on the noted clinical responses in patients being treated. In one study of 67 patients with lentigo maligna melanoma, 45 out of 67 (67%) achieved a complete remission, 5 out of 67 (7%) had a partial response to therapy and 8 out of 67 (12%) failed to respond. Although seemingly effective against non-invasive melanoma *in situ* and other cutaneous lesions, it is unclear as to the ultimate effect it may have on more invasive melanoma lesions that will be more effectively treated with adequate surgical

excision. The few examples of imiquimod therapy for metastatic melanoma have used a combinatorial approach with other agents, such as melphalan and pulsed-dye lasers, noting a select few patients that had an objective response to therapy and rare complete remission [80-82].

## 7. Plasmid and recombinant DNA vaccines

A rapidly developing area of immunotherapy involves the use of naked plasmid DNA, or recombinant viral vectors that encode tumor-associated antigens. Activation of cytotoxic T lymphocytes (CTLs) can occur via two pathways: the first involves the direct transfection or transduction of APCs by the DNA vaccine and in this case, the protein antigen is processed through the endogenous pathway for presentation to and priming of CTLs [83,84]; the second pathway involves the transfection or transduction of non-APCs, such as skeletal muscle cells, in which the protein antigen is expressed, taken up and processed by APCs for presentation to CTLs via a process called cross-presentation [85,86]. Preclinical murine models using intramuscular injection of plasmid DNA have demonstrated long-term protein expression of the gene of interest [87,88].

There are several advantages in using naked plasmid DNA as a vaccine. These include the simplicity and relatively low cost of production, in addition to the plasmid backbone containing unmethylated CpG motifs that are able to activate DC via the TLR-9 receptor [89,90]. An additional advantage is the ability of DNA vaccines to induce the activation of CD4<sup>+</sup>, CD8<sup>+</sup> T cells and B cells for effective cellular and humoral immunity. In a Phase I study of stage IV melanoma patients treated with plasmid DNA encoding the gp100 melanoma differentiation antigen, only 1 out of 22 (5%) developed a partial response with none showing evidence of immunologic activation [91]. In another study using plasmid DNA encoding the melanoma antigen recognized by T cells (MART)-1 antigen to treat patients with resected melanoma at high risk for recurrence, analysis of post-vaccination peripheral blood mononuclear cells did not show any changes in immune responses to the MART-1 antigen [92].

Another DNA vaccine approach uses viral vectors as carriers for the cDNA encoding the gene of interest. The advantage of using recombinant viruses to carry the gene encoding for an antigen is that viruses can efficiently infect cells, particularly DCs [93]. Although appealing to use for multiple reasons, several viral vectors are known to induce a tremendous antiviral neutralizing antibody response to the first and subsequent vaccinations, severely limiting the effectiveness of this approach. One exception may be the use of fowlpox viral vectors, which do not seem to produce neutralizing antibodies. Several studies have been performed using adenovirus, vaccinia, canarypox or fowlpox viruses encoding various melanoma antigens, such as gp100, MART-1, tyrosinase or melanoma antigen gene, with only occasional immunologic or clinical responses observed [94-96].

In a recent study, recombinant vaccinia and fowlpox encoding the antigen NY-ESO-1 used in a prime-boost vaccination setting were found to induce humoral and/or cellular responses in most patients [96]. Kaufman *et al.* [97-99] have examined the use of various combinations of a recombinant vaccinia virus (vaccinia/TRICOM, recombinant fowlpox/B7.1 and fowlpox/TRICOM expressing the human *B7.1* gene) in patients with unresectable melanoma, which were designed to evaluate the toxicity of the vaccine, the ability to generate melanoma-specific immunity and the therapeutic efficacy. However, despite the use of a plethora of different viral vectors encoding different melanoma-associated antigens and costimulatory molecules, the results have been equally disappointing. Rosenberg *et al.* [5] describe > 160 patients treated with various viral vaccines, with only 2 of 160 developing any clinical response (1 partial and 1 complete response). The overall objective response rate for the various viral vaccination strategies was found to be only 1.9%.

## 8. Adoptive cell transfer therapy

Adoptive cell transfer therapy for melanoma patients initially involves the isolation and growth of large numbers of highly active, melanoma-specific autologous T cells *in vitro*. This is followed by infusion of these reactive T cells back into the same patient, usually also given in combination with high-dose IL-2. This approach represents a promising therapy for the treatment of advanced melanoma [100]. The initial studies using cloned melanoma-antigen specific T cells, with or without IL-2, seemed to be ineffective in inducing an objective antitumor response [101,102]. Another approach is the use of tumor-infiltrating lymphocytes (TILs), which has the hypothetical advantage of containing a diverse effector population composed of both CD4<sup>+</sup> and CD8<sup>+</sup> T cells. In clinical studies in which TILs were used in conjunction with high-dose IL-2, objective tumor regressions were seen in 33% of the patients treated, but, unfortunately, these clinical responses were of short duration with the persistence of the transferred TILs transient [103,104].

To enhance the efficacy of adoptive cell transfer using TILs, a lymphodepleting, non-myeloablative preconditioning chemotherapy regimen consisting of cyclophosphamide and fludarabine was given before the administration of highly reactive melanoma-antigen specific TILs combined with high dose IL-2 [105]. The initial study involved 13 patients with progressive melanoma despite multiple previous treatments, including high-dose IL-2, the results showed that 6 out of 13 patients achieved an objective clinical responses and 4 out of 13 exhibiting a mixed response there was regression of some lesions, but with progression in other lesions [105]. In a recent follow-up study, cancer regression in patients with refractory metastatic melanoma with large, vascularized tumors was noted in a striking 18 out of 35 patients (51% response rate), including 4 patients with a complete regression of all metastatic disease [106]. This is the highest

reported response rate for the immunotherapeutic treatment of patients with stage IV melanoma to date.

This is an exciting and dynamic era for the development of cutting-edge discoveries and research focused on melanoma immunotherapy. It is clear that highly reactive cytotoxic T cells are indeed capable of mediating the destruction of large, bulky tumors and, rarely, rendering stage IV melanoma patients disease free. Recently, the Rosenberg group [107] examined the use of autologous peripheral blood lymphocytes transduced with a retrovirus encoding a T cell receptor for the MART-1 antigen *ex vivo*. This experimental regimen was administered to a total of 15 patients in conjunction with high-dose IL-2 in a lymphodepleting, non-myeloablative setting. A total of 2 out of 15 patients achieved objective clinical responses that are ongoing after 1 year, demonstrating for the very first time the efficacy of gene therapy combined with a multimodal approach (IL-2, non-myeloablative chemotherapy) in the treatment of stage IV melanoma.

The underlying immunologic and cellular mechanisms of such a lymphodepleting preconditioning regimen are not fully understood. However, there are several factors that may contribute to the enhanced efficacy of the adoptively transferred T cells: the elimination of cellular cytokine 'sinks' for homeostatic cytokines such as IL-7, IL-15 and possibly IL-21, which promote activation and proliferation of melanoma-reactive T cells; the elimination of immunosuppressive cells, such as Tregs; and the activation of APCs by the preconditioning regimen [108].

### 9. Targeted therapy for melanoma

Targeted therapy for patients with metastatic melanoma has developed rapidly over the last 10 years as knowledge has grown in regard to specific targets and immunologic breakthroughs. For example, although Tregs have been realized for some time, their key role in potential tumor-escape mechanisms has only recently been discovered. Curiel *et al.* [109] have identified a potent tumor-induced escape mechanism for patients with advanced ovarian cancer whereby the tumor cells are able to produce a chemokine (CCL22) capable of attracting regulatory T cells to the tumor site and malignant ascities. This group also showed that as the number of Tregs increased at the tumor site, the overall survival for these patients decreased dramatically, highlighting the powerful immunosuppressive effects of the Tregs on the host immune system. Thus, several researchers have identified the potential importance of suppressing and/or eliminating Tregs as a first step in an effective treatment strategy for patients with advanced melanoma.

Tregs are known to play a critical role in immune tolerance, autoimmunity and suppression of the host immune response to cancer [110-117]. Denileukin diftitox is an immunotoxin that is specific to the IL-2 receptor. Denileukin diftitox has FDA approval for treating patients with cutaneous T cell

lymphoma, with an overall response rate in the 30 – 40% range [118]. Recently, Dunnell *et al.* [119] have shown that denileukin diftitox induced elimination of Tregs followed by vaccination with RNA-transfected DC significantly improves the stimulation of tumor-specific T cell responses in renal cell carcinoma patients. However, compelling data from Attia *et al.* has revealed a much different conclusion as to the ability of denileukin diftitox to eliminate Tregs [120]. They have extensively examined the functional use of denileukin diftitox, failing to demonstrate that it was capable of eliminating the absolute number of Tregs or decreasing their suppressive activity. Thus, the present use of denileukin diftitox in modulating Tregs in cancer patients remains a question at this time.

There have been numerous studies that have implicated the central role of cellular signaling through the RAF/MEK/ERK/MAPK and its involvement in melanocytic tumorigenesis. Sorafenib is an orally administered multikinase inhibitor, which not only targets BRAF serine/threonine kinases that are frequently mutated in melanoma, but also targets several tyrosine kinases receptors associated with tumor angiogenesis and progression. Despite strong preclinical data, a recent randomized Phase II trial demonstrated that sorafenib monotherapy had little or no antitumor activity in advanced melanoma patients [121]. Interestingly, there was no apparent correlation between the presence of an oncogenic V600E BRAF mutation within exon 15 and the modest antitumor activity of sorafenib monotherapy. Others then attempted to combine sorafenib with other chemotherapeutic agents that had some activity against melanoma, such as carboplatin and paclitaxel. This combination induced one complete response (< 1%), 27 partial response (26%) and 61 disease stabilizations (58%) in patients with advanced melanoma [122]. In another Phase I trial, the combination of sorafenib with dacarbazine induced a partial response in 3 and disease stabilizations in 5 out of 10 evaluable patients with advanced melanoma [123]. Further investigations are being actively pursued in combining sorafenib with other cytotoxic agents.

Antisense oligonucleotides are short sequences of cDNA engineered to bind to specific gene sequences and prevent the translation of mRNA, with resultant abrogation of protein synthesis. It has been shown that one mechanism of melanoma cell survival is due to the overexpression of the *Bcl-2* gene, which produces an antiapoptotic protein that blocks the release of cytochrome c. The downregulation of the Bcl-2 protein results in a higher level of chemotherapy-induced apoptosis in human cancer xenografts [124]. A recent randomized Phase III trial compared dacarbazine alone with dacarbazine plus oblimersen, a Bcl-2 antisense oligonucleotide [125]. This large, randomized trial of 771 patients revealed that there was a trend toward improved survival at 24-months of follow up (median: 9 versus 7.8 months;  $p = 0.077$ ) with significant differences in progression-free survival (median: 2.6 versus 1.6 months), overall response (13.5 versus 7.5%), complete response (2.8 versus 0.8%) and durable response (7.3 versus 3.6%).

Interestingly, the 508 patients with a normal lactate dehydrogenase (LDH) out of a total of 771 had a significantly improved overall survival with the combined therapy (11.4 versus 9.7 months). Thus, this study suggests that LDH can be used as a pretreatment biomarker, confirming that there is an overall poor prognosis for those patients with an elevated LDH prior to treatment. Furthermore, the authors suggest that the serum LDH should be used to identify patients who are unlikely to benefit from oblimersen-dacarbazine treatment. Although the survival differences are marginal, it does reveal that oblimersen has clear and significant activity in melanoma. Future trials with oblimersen and other such agents should include patients only with a normal LDH, maximizing the chances that such agents will have a significant activity and/or impact on patient survival, without the diluting effect of patients with more advanced/aggressive disease and an elevated LDH.

Another novel approach to the treatment of patients with metastatic melanoma involves the use of different antibodies to reduce the sensitivity of activated T cells to negative regulatory signals. One mechanism of immune regulation is via cytotoxic T-lymphocyte antigen (CTLA)-4 protein expression on activated T cells, transducing signals back to the T cells that inhibit T cell activation, proliferation and IL-2 production. It has also been shown that the CTLA-4 receptor is present on Tregs, with recent controversial data on whether CTLA-4 blockade leads to the depletion or impairment of Tregs.

The human monoclonal anti-CTLA-4 antibody CP-675206 is an IgG2 antibody with high affinity for CTLA-4 on the surface of T cells. Ribas *et al.* [126] recently conducted a Phase I clinical trial in 34 patients with stage IV melanoma treated with a single dose of this antibody. There were 4 out of 34 (12%) patients with objective responses, 2 patients with a complete regression of all disease and two others with a partial response. An additional 4 patients were considered to have stable disease. Autoimmune side effects were considerable, and included vitiligo, dermatitis, thyroiditis, hypophysitis and colitis.

A second anti-CTLA-4 antibody, MDX-010, has also been tested in early phase clinical trials. Hersh *et al.* [127] presented their preliminary results of a randomized Phase II trial of 76 patients randomized to MDX-010 injections once per month alone (for 4 months) or combined with dacarbazine. A total of 2 out of 76 patients had partial responses to treatment with the antibody alone and 5 out of 76 patients responding to the combination therapy. These early results suggest that the MDX-010 antibody has efficacy in melanoma as a monotherapy and possibly improved effect with combinatorial therapy, such as vaccines, cytokines or growth factors.

In a recent Phase I/II study of 36 heavily pretreated patients with stage IV melanoma, 8 out of 36 (22%) experienced an objective tumor response (3 complete responses and 5 partial responses), with all patients receiving concomitant high-dose IL-2 therapy [128]. Although there was no evidence of a synergistic effect between the antibody and IL-2, there was a

correlation between tumor regression and the development of autoimmune phenomenon, such as vitiligo, a finding supported by previous studies [129-131].

## 10. Electrochemotherapy and electroimmunotherapy

Electroporation has been used for > 20 years as a means to facilitate transport of normally non-permeant molecules into cells. This technique has been used to allow dyes, DNA, RNA, ions, drugs and proteins into cells *in vitro* [132]. Radiotracers, drugs and modified deoxoligonucleotides have been loaded into cells *in vivo* [133-136]. Thus, electrochemotherapy (ECT) has evolved as a fairly novel form of anticancer therapy in which transient permeabilization of cells by an electric field induces a significant increase in the concentration of chemotherapeutic agents and subsequent toxicity in tumor cells. Rols *et al.* [137] used ECT to administer bleomycin within the skin at subcutaneous tumor sites, noting antitumor effects in > 90% of the 55 nodules treated and a complete response rate of 9%. Several other studies have assessed the efficacy of ECT in the treatment of metastatic melanoma with various chemotherapeutic agents and cytokines, such as bleomycin, cisplatin and IL-12 [138-143].

The Sydney Melanoma Unit has recently completed a Phase II, randomized, open-label, crossover study of 19 patients receiving either a combination of intralesional bleomycin plus electroporation therapy versus bleomycin alone [144]. They found the combination to be significantly more effective in the treatment of metastatic melanoma compared with intralesional bleomycin alone. ECT provides a non-invasive therapeutic option for melanoma patients. In order to increase its efficacy, ECT can be combined with other options, such as immunotherapy.

## 11. Surgery as an adjunctive strategy to immunotherapy

Although surgical removal of metastatic disease has been performed for centuries, it is only recently that a scientific explanation and hypothesis has been applied to the occasional observation that surgical removal of metastatic disease can result in prolonged survival and rare cures. It has been hypothesized that the surgical removal of bulky macroscopic disease may allow the host immune response to overcome tumor-induced immunosuppression, such as the direct secretion of IL-10, prostaglandin E<sub>2</sub>, TGF- $\beta$  and growth factors, such as vascular endothelial growth factor, into the tumor microenvironment [36]. Thus the removal of large tumors leaving only microscopic disease allows for a 'resetting' of the immune clock, providing a much smaller volume of tumor to be dealt with by the previously tolerized host immune system [36].

Further evidence in support of a role for complete surgical removal of all metastatic disease to microscopic levels only is

derived from the recent onamelatucel-L trials, designed to assess the efficacy of this vaccine in both stage III and IV melanoma patients. All patients in the stage IV trial underwent definitive complete cytoreductive surgery prior to being randomized. This trial was closed early because it was found on interim analysis that the vaccine was no better than placebo. However, it was interesting to find that all patients (regardless of whether they were randomized to the control arm or canvaxin arm) had a higher 5-year survival rate of 40% compared with historical controls, suggesting that prolonged survival was not due to the vaccine, but rather the surgical removal of metastatic disease [35,36,145]. Others have reported durable disease-free and long-term survival in patients with stage IV melanoma after resection of all metastatic disease which was either previously treated with or followed by some form of systemic-based immunotherapy [146,147].

## 12. Expert opinion

The research efforts directed towards the development of novel therapies for patients with metastatic melanoma have been intense despite disappointing clinical outcomes. It is clear that monotherapy for metastatic melanoma will not be successful, with every study to date yielding minimal clinical outcomes data. Therefore, we must realize that there is no magic bullet to cure melanoma, and we must develop multimodality strategies to enhance the immune response to melanoma from different angles. This will require an unprecedented

collaborative effort by many in order to overcome the historical competition between large pharmaceutical companies striving for 'total cure' with their drug.

We should realize that the future of drug development and design will depend heavily on the recent trend towards molecular medicine, and in particular, gene profiling efforts using gene microarray analysis. We are entering an entire new field of research dedicated to the molecular basis of cancer. Such research has the greatest potential to impact the way we treat patients with melanoma, focusing results on the prognostic significance of particular genes from a melanoma patient and basing clinical decisions as to whether such patients will (or will not) respond to a particular agent. The development of molecular signatures using gene microarray analysis has come to the forefront of existing research efforts identifying patients who may have an aggressive (versus indolent) form of melanoma and those patients with particular prognostic gene signatures that may predict the response to forms of immunotherapy. The identification of such gene signatures has had important implications in the development of targeted immunotherapies for patients with metastatic disease. Thus, we must focus our efforts towards an improved understanding of the molecular and immunologic events involved in melanoma development and progression. We should also re-evaluate our present approach to immunotherapy and trial design, with many past trials failing to show clinical efficacy because of a lack of appropriate preclinical data that provide the essential rationale to perform such studies.

### Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. BALCH CM, BUZAID AC, SOONG MB *et al.*: New TNM melanoma staging system: linking biology and natural history to clinical outcomes. *Semin. Surg. Oncol.* (2003) 21:43-52.
2. BALCH CM, BUZAID AC, SOONG SJ *et al.*: Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. *Cancer* (2001) 88:3635-3648.
3. BALCH CM, SOONG SJ, GERSHENWALD JE *et al.*: Prognostic factors analysis of 17,600 melanoma patients. Validation of the American Joint Committee on Cancer melanoma staging system. *J. Clin. Oncol.* (2001) 19:3622-3634.
4. ATALLAH E, FLAHERTY L: Treatment of metastatic malignant melanoma. *Curr. Treat. Options Oncol.* (2005) 6:185-193.
5. ROSENBERG SA, YANG JC, RESTIFO NP: Cancer immunotherapy: moving beyond current vaccines. *Nat. Med.* (2004) 10:909-915.
- A critical and sobering look at the low overall response rates for clinical trials of patients with metastatic cancer (mainly melanoma) over the last decade that have been treated with some form of an immunotherapeutic vaccine.
6. KIRKWOOD JM: Cancer immunotherapy: the interferon- $\alpha$  experience. *Semin. Oncol.* (2002) 29:18-26.
7. KIRKWOOD JM, STRAWDERMAN MH, ERNSTOFF MS, SMITH TJ, BORDEN EC, BLUM RH: Interferon  $\alpha$ -2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684. *J. Clin. Oncol.* (1996) 14:7-17.
8. KIRKWOOD JM, IBRAHIM JG, SONDAK VK *et al.*: High- and low-dose interferon  $\alpha$ -2b in high-risk melanoma: first analysis of intergroup trial E1690/S9111/C9190. *J. Clin. Oncol.* (2000) 18:2444-2458.
9. KIRKWOOD JM, IBRAHIM JG, SOSMAN JA *et al.*: High-dose interferon  $\alpha$ -2b significantly prolongs relapse-free and overall survival compared with the GM2-KLH/QS-21 vaccine in patients with resected stage IIB-III melanoma: results of intergroup trial, E1694. *J. Clin. Oncol.* (2001) 19:2370-2380.
10. WHEATLEY K, IVES N, HANCOCK B, GORE M, EGGERMONT A, SUCIU S: Does adjuvant interferon- $\alpha$  for high-risk melanoma provide a worthwhile benefit? A meta-analysis of the randomised trials. *Cancer Treat. Rev.* (2003) 29:241-252.
- This is one of the most thorough meta-analyses to date discussing the statistical outcomes of patients treated with all forms of IFN- $\alpha$  (high, intermediate and low).

11. ROSENBERG SA, YANG JC, TOPALIAN SL *et al.*: Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high-dose bolus interleukin-2. *JAMA* (2004) 271:907-913.
12. ATKINS MB, LOTZE MT, DUTCHER JP *et al.*: High-dose recombinant interleukin-2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. *J. Clin. Oncol.* (1999) 17:2105-2116.
13. ATKINS MB, KUNKEL L, SZNOL M, ROSENBERG SA: High-dose recombinant interleukin-2 therapy in patients with metastatic melanoma: long-term survival update. *Cancer J. Sci. Am.* (2000) 6:S11-S14.
- A review of patients treated with high-dose IL-2, showing the durability of the complete responders.
14. SPANKNEBEL K, CHEUNG KY, STOUTENBURG MD *et al.*: Initial clinical response predicts outcome and is associated with dose schedule in metastatic melanoma and renal cell carcinoma patients treated with high-dose interleukin-2. *Ann. Surg. Oncol.* (2005) 12:1-10.
15. QUAN W JR, BRICK W, VINOGRADOV M, TAYLOR WC, KHAN N, BURGESS R: Repeated cycles with 72-hour continuous infusion interleukin-2 in kidney cancer and melanoma. *Cancer Biother. Radiopharm.* (2004) 19:350-354.
16. PIN J, WANG E, PROVENZANO M *et al.*: Molecular signatures induced by interleukin-2 on peripheral blood mononuclear cells and T-cell subsets. *J. Trans. Med.* (2006) 4:1-23.
17. PANELLI MC, WAND E, PHAN G *et al.*: Genetic profiling of peripheral mononuclear cells and melanoma metastases in response to systemic interleukin-2 administration. *Genome Biol.* (2002) 3:1-17.
18. ATKINS MB, BUZAID AC, HOUGHTON AN: Chemotherapy and biochemotherapy. In: *Cutaneous Melanoma. 4th edn.* Balch C, Houghton A Jr, Sober A, Soong S (Eds), Quality Medical Publishing, Inc., St Louis, MO, USA (2003):589-604.
19. MCDERMOTT DF, MIER JW, LAWRENCE DP *et al.*: A Phase II pilot trial of concurrent biochemotherapy with cisplatin, vinblastine, dacarbazine, interleukin 2, and interferon-2B in patients with metastatic melanoma. *Clin. Cancer Res.* (2000) 6:2201-2208.
20. FLAHERTY LE, ATKINS M, SOSMAN J *et al.*: Outpatient biochemotherapy with interleukin-2 and interferon  $\alpha$ -2b in patients with metastatic malignant melanoma: results of two Phase II Cytokine Working Group trials. *J. Clin. Oncol.* (2001) 19:3194-3202.
21. KEILHOLZ U, GOEY SH, PUNT CJ *et al.*: Interferon-2a and interleukin-2 with or without cisplatin in metastatic melanoma: a randomized trial of the European Organization for Research and Treatment of Cancer Melanoma Cooperative Group. *J. Clin. Oncol.* (1997) 15:2579-2588.
22. ROSENBERG SA, YANG JC, SCHWARTZENTRUBER DJ *et al.*: Prospective randomized trial of the treatment of patients with metastatic melanoma using chemotherapy with cisplatin, dacarbazine, and tamoxifen alone or in combination with interleukin-2 and interferon  $\alpha$ -2b. *J. Clin. Oncol.* (1999) 17:968-975.
23. ETON O, LEGHA SS, BEDIKIAN AY *et al.*: Sequential biochemotherapy versus chemotherapy for metastatic melanoma: results from a Phase III randomized trial. *J. Clin. Oncol.* (2002) 20:2045-2052.
24. ATKINS MB, LEE S, FLAHERTY LE, SOSMAN JA, SONDAK VK, KIRKWOOD JM: A prospective randomized Phase III trial of concurrent biochemotherapy (BCT) with cisplatin, vinblastine, dacarbazine (CVD), IL-2 and interferon-2b (IFN) versus CVD alone in patients with metastatic melanoma (E3695): an ECOG-coordinated intergroup trial. In: *Program and Abstracts of the American Society of Clinical Oncology Annual Meeting.* Chicago (IL) USA (31 May – 3 June 2003):708a.
25. PUNT CJ, SUCIU S, GORE MA *et al.*: Chemoimmunotherapy with dacarbazine, cisplatin, interferon- $\alpha$ 2b and interleukin-2 versus two cycles of dacarbazine followed by chemoimmunotherapy in patients with metastatic melanoma: a randomized Phase II study of the EORTC. *Eur. J. Cancer* (2006) 42(17):2991-2995.
26. BERD D, MAGUIRE HC, MASTRANGELO MJ: Induction of cell-mediated immunity to autologous melanoma cells and regression of metastases after treatment with a melanoma cell vaccine preceded by cyclophosphamide. *Cancer Res.* (1986) 46:2572-2577.
27. LIVINGSTON PO, CUNNINGHAM-RUNDLES S, MARFLEET G *et al.*: Inhibition of suppressor-cell activity by cyclophosphamide in patients with malignant melanoma. *J. Biol. Response Modif.* (1987) 6:392-403.
28. BERD D, MAGUIRE HC JR, MCCUE P, MASTRANGELO MJ: Treatment of metastatic melanoma with an autologous tumor-cell vaccine: clinical and immunologic results in 64 patients. *J. Clin. Oncol.* (1990) 8:1858-1867.
29. LIVINGSTON PO, WONG GY, ADLURI S *et al.*: Improved survival in stage III melanoma patients with GM2 antibodies: a randomized trial of adjuvant vaccination with GM2 ganglioside. *J. Clin. Oncol.* (1994) 12:1036-1044.
30. BYSTRYN JC, ORATZ R, HARRIS MN, ROSES DF, GOLOMB FM, SPEYER JL: Immunogenicity of a polyvalent melanoma antigen vaccine in humans. *Cancer* (1988) 61:1065-1070.
31. BERD D, SATO T, MAGUIRE HC, KAIRYS J, MASTRANGELO MJ: Immunopharmacologic analysis of an autologous, hapten-modified human melanoma vaccine. *J. Clin. Oncol.* (2004) 22:403-415.
32. GHIRINGHELLI F, LARMONIER N, SCHMITT E *et al.*: CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. *Eur. J. Immunol.* (2004) 34:336-344.
33. ERCOLINI AM, LADLE BH, MANNING EA *et al.*: Recruitment of latent pools of high-avidity CD8-positive T cells to the anti-tumor immune response. *J. Exp. Med.* (2005) 201:1591-1602.

34. RADFAR S, WANG Y, KHONG HT: 'Chemocentric' chemoimmunotherapy: presensitization of tumor cells with activated CD4<sup>+</sup> T cells synergistically enhances the cytotoxic effect of chemotherapy. *J. Immunother.* (2006) **29**(6):632.
- Preliminary work revealing a novel approach to immunotherapy via the activation of CD4<sup>+</sup> T cells.
35. MORTON DL: Surgery prolongs survival in stage IV melanoma. *Symposium of the Society of Surgical Oncology*. San Diego, California, USA (23 – 26 March 2006).
36. MORTON DL, OLLILA DW, HSUEH EC, ESSNER R, GUPTA RK: Cytoreductive surgery and adjuvant immunotherapy: a new management paradigm for metastatic melanoma. *CA Cancer J. Clin.* (1999) **49**:101-116.
37. YOUNG SE, MARTINEZ SR, FARIES MB, ESSNER R, WANER LA, MORTON DL: Can surgical therapy alone achieve long-term cure of melanoma metastatic to the regional nodes. *Cancer J.* (2006) **12**:207-211.
38. HSUEH EC, MORTON DL: Antigen-based immunotherapy of melanoma: Canvaxin therapeutic polyvalent cancer vaccine. *Semin. Cancer Biol.* (2003) **13**:401-407.
39. ELLIOT GT, MCLEOD RA, PEREZ J, VON ESCHEN KB: Interim results of a Phase II multicenter clinical trial evaluating the activity of a therapeutic allogeneic melanoma vaccine in the treatment of disseminated malignant melanoma. *Semin. Surg. Oncol.* (1993) **2**:41-53.
40. MITCHELL MS, VON ESCHEN KB: Phase III trial of Melacine melanoma theraccine versus combination chemotherapy in the treatment of stage IV melanoma. *Proc. Am. Soc. Clin. Oncol.* (1997) **16**:494-499.
41. SONDAK VK, LIU PY, TUTHILL RJ *et al.*: Adjuvant immunotherapy of resected, intermediate-thickness node-negative melanoma with an allogeneic tumor vaccine. I. Overall results of a randomized trial of SWOG. *J. Clin. Oncol.* (2002) **20**:2058-2066.
- One of the largest randomized trials to date involving the use of an allogeneic tumor cell-based vaccine and associated clinical outcomes.
42. SONDAK VK, SOSMAN JA: Results of clinical trials with an allogeneic melanoma tumor cell lysate vaccine: (Melacine). *Semin. Cancer Biol.* (2003) **13**:409-415.
43. DILLMAN RO, DELEON C, BEUTEL LD *et al.*: Short-term autologous tumor cell lines for the active specific immunotherapy of patients with metastatic melanoma. *Crit. Rev. Oncol. Hematol.* (2001) **39**:115-123.
44. DILLMAN RO, BEUTEL LD, BARTH NM *et al.*: Irradiated cells from autologous tumor cell lines as patient-specific vaccine therapy in 125 patients with metastatic cancer: induction of delayed-type hypersensitivity to autologous tumor is associated with improved survival. *Cancer Biother. Radiopharm.* (2002) **17**:51-66.
45. HOOVER HC, SURDYKE M, DANGEL RB, PETERS LC, HANNA MG: Delayed cutaneous hypersensitivity to autologous tumor cells in colorectal cancer patients immunized with an autologous tumor cell-BCG vaccine. *Cancer Res.* (1984) **44**:1671-1676.
46. BYSTRYN JC, ORATZ R, ROSES D, HARRIS M, HENN M, LEW R: Relationship between immune response to melanoma vaccine immunization and clinical outcome in stage II malignant melanoma. *Cancer* (1992) **69**:1157-1164.
47. BARTH A, HOON DS, FOSHAG LJ *et al.*: Polyvalent melanoma cell vaccine induces a delayed-type hypersensitivity and *in vitro* cellular immune response. *Cancer Res.* (1994) **54**:3342-3345.
48. MILLER K, ABELES G, ORATZ R *et al.*: Improved survival of patients with melanoma with an antibody response to immunization to a polyvalent melanoma vaccine. *Cancer* (1995) **75**:495-502.
49. BERD D, MAGUIRE HC, SCHUCHTER LM *et al.*: Autologous hapten modified melanoma vaccine as a post surgical adjuvant treatment after resection of nodal metastases. *J. Clin. Oncol.* (1997) **15**:2359-2370.
50. BERD D, SATO T, COHN H, MAGUIRE HC, MASTRANGELO MJ: Treatment of metastatic melanoma with autologous hapten-modified melanoma vaccine: Regression of pulmonary metastases. *Int. J. Cancer* (2001) **94**:531-539.
51. LOTEM M, PERETZ T, DRIZE O *et al.*: Autologous cell vaccine as a post operative adjuvant treatment for high-risk melanoma patients (AJCC III and IV). *Br. J. Cancer* (2002) **86**:1534-1539.
52. DRANOFF G, JAFFEE E, LAZENBY A *et al.*: Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc. Natl. Acad. Sci. USA* (1993) **90**:3539-3543.
53. MACH N, GILLESSEN S, WILSON SB *et al.*: Differences in dendritic cells stimulated *in vivo* by tumors engineered to secrete granulocyte-macrophage colony-stimulating factor or Flt3-ligand. *Cancer Res.* (2000) **60**:3239-3246.
54. CHANG AE, LI Q, BISHOP DK *et al.*: Immunogenetic therapy of human melanoma utilizing autologous tumor cells transduced to secrete granulocyte-macrophage colony-stimulating factor. *Hum. Gene Ther.* (2000) **11**:839-850.
55. SOIFFER R, HODI FS, HALUSKA F *et al.*: Vaccination with irradiated, autologous melanoma cells engineered to secrete granulocyte-macrophage colony-stimulating factor by adenoviral-mediated gene transfer augments antitumor immunity in patients with metastatic melanoma. *J. Clin. Oncol.* (2003) **21**:3343-3350.
56. LUITEN RM, KUETER EW, MOOI W *et al.*: Immunogenicity, including vitiligo, and feasibility of vaccination with autologous GM-CSF-transduced tumor cells in metastatic melanoma patients. *J. Clin. Oncol.* (2005) **23**:8978-8991
57. ROSENBERG SA, YANG JC, SCHWARTZENTRUBER DJ *et al.*: Immunologic and therapeutic evaluation of a synthetic tumor associated peptide vaccine for the treatment of patients with metastatic melanoma. *Nat. Med.* (1998) **4**:321-327.
- Seminal article revealing the immunologic activation of the immune system in response to a peptide vaccine.
58. RIKER AI, PANELLI M, KAMMULA US *et al.*: Threshold levels of gene expression of the melanoma antigen, gp100, correlates with tumor cell recognition by cytotoxic T-lymphocytes. *Int. J. Cancer* (2000) **86**:818-826.

59. RIKER AI, PANELLI M, KAMMULA US *et al.*: Development and characterization of melanoma cell lines established by fine needle aspiration biopsy: advances in the monitoring of patients with metastatic melanoma. *Cancer Detect. Prev.* (1999) **23**:387-396.
60. SLINGLUFF CL, PETRONI GR, YAMSHCHIKOV GV *et al.*: Clinical and immunologic results of a randomized Phase II trial of vaccination using four melanoma peptides either administered in granulocyte-macrophage colony-stimulating factor in adjuvant or pulsed on dendritic cells. *J. Clin. Oncol.* (2003) **21**:4016-4026.
61. SLINGLUFF CL, CHIANESE-BULLOCK KA, BULLOCK TN *et al.*: Immunity to melanoma antigens: from self-tolerance to immunotherapy. *Adv. Immunol.* (2006) **90**:243-295.
62. RIDGEWAY D: The first 1,000 dendritic cell vaccines. *Cancer Invest.* (2003) **21**:876-886.
- **Thorough review of the history of DC-based immunotherapy for patients with advanced melanoma.**
63. SCHADENDORF D, UGUREL S, SCHULER-TURNER B *et al.*: Dacarbazine (DTIC) versus vaccination with autologous peptide-pulsed dendritic cells (DC) in first-line treatment of patients with metastatic melanoma: a randomized Phase III trial of the DC study group of the DeCOG. *Ann. Oncol.* (2006) **17**:563-570.
64. PALUCKA AK, UENO H, CONNOLLY J *et al.*: Dendritic cells loaded with allogeneic melanoma cells induce objective clinical responses and MART-1 specific CD8<sup>+</sup> T-cell immunity. *J. Immunother.* (2006) **29**:545-557.
65. NESTLE FO, ALIJAGIC S, GILLIET M *et al.*: Vaccination of melanoma patients with peptide-or tumor lysate-pulsed dendritic cells. *Nat. Med.* (1998) **4**:328-332.
- **One of the first DC vaccine trials showing true clinical responders (both partial and complete) to therapy.**
66. CHANG AE, REDMAN BG, WHITFIELD J *et al.*: A Phase I trial of tumor lysate-pulsed dendritic cells in the treatment of advanced cancer. *Clin. Cancer Res.* (2002) **8**:1021-1032.
67. NAGAYAMA H, SATO K, MORISHITA M *et al.*: Results of a Phase I clinical study using autologous tumor lysate-pulsed monocyte-derived mature dendritic cell vaccinations for stage IV malignant melanoma patients combined with low dose interleukin-2. *Melanoma Res.* (2003) **13**:521-530.
68. KRIEG AM: Therapeutic potential of Toll-like receptor 9 activation. *Nat. Rev. Drug Discov.* (2006) **5**:471-484.
69. KRIEG AM: CpG motifs in bacterial DNA: the active ingredients in BCG and CFA? *Nat. Med.* (2003) **9**:831-835.
70. HECKELSMILLER K, BECK S, RALL K *et al.*: Combined dendritic cell- and CpG oligodeoxynucleotide- based immune therapy cures large murine tumors that resist chemotherapy. *Eur. J. Immunol.* (2002) **32**:3235-3245.
71. SPEISER DE, LIENARD D, RUFER N *et al.*: Rapid and strong human CD8<sup>+</sup> T cell responses to vaccination with peptide, IFA, and CpG oligodeoxynucleotide 7909. *J. Clin. Invest.* (2005) **115**:739-746.
72. SANDLER AD, CHIHARA H, KOBAYASHI G *et al.*: CpG oligodeoxynucleotides enhance the tumor antigen-specific immune response of a granulocyte-macrophage colony-stimulating factor-based vaccine strategy in neuroblastoma. *Cancer Res.* (2003) **63**:394-399.
73. DAVILA HL, CELIS E: Repeated administration of cytosine-phosphorothiolated guanine-containing oligonucleotides together with peptide/protein immunization results in enhanced CTL responses with anti-tumor activity. *J. Immunol.* (2000) **165**:539-547.
74. KAWARADA Y, GANSS R, GARBI N, SACHER T, ARNOLD B, HAMMERLING GJ: NK- and CD8<sup>+</sup> T-cell mediated eradication of established tumors by peritumoral injection of CpG-containing oligodeoxynucleotides. *J. Immunol.* (2001) **167**:5247-5253.
75. VAN OJIK HH, BEVAART L, DAHLE CE *et al.*: CpG-A and -B oligodeoxynucleotides enhance the efficacy of antibody therapy by activating different effector cell populations. *Cancer Res.* (2003) **63**:5595-5600.
76. TORMO D, FERRER A, BOSCH P *et al.*: Therapeutic efficacy of antigen-specific vaccination and Toll-like receptor stimulation against established transplanted and autochthonous melanoma in mice. *Cancer Res.* (2006) **66**:5427-5435.
77. MOSEMAN EA, LIANG X, DAWSON AJ *et al.*: Human plasmacytoid dendritic cells activated by CpG oligodeoxynucleotides induce the generation of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells. *J. Immunol.* (2004) **173**:4433-4442.
78. RAJPAR SF, MARSDEN JR: Imiquimod in the treatment of lentigo maligna. *Br. J. Dermatol.* (2006) **155**:653-656.
79. WOLF IH, SMOLLE J, BINDER B *et al.*: Topical imiquimod in the treatment of metastatic melanoma to skin. *Arch. Dermatol.* (2003) **139**:273-276.
80. UTIKAL J, ZIMPFER A, THOELKE A *et al.*: Complete remission of multiple satellite and in-transit melanoma metastases after sequential treatment with isolated limb perfusion and topical imiquimod. *Br. J. Dermatol.* (2006) **155**:488-491.
81. ZEITOUNI NC, DAWSON K, CHENEY RT: Treatment of cutaneous metastatic melanoma with imiquimod 5% cream and the pulsed-dye laser. *Br. J. Dermatol.* (2005) **152**:376-377.
82. CRAFT N, BRUHN KW, NGUYEN BD *et al.*: The TLR7 agonist imiquimod enhances the anti-melanoma effects of a recombinant *Listeria monocytogenes* vaccine. *J. Immunol.* (2005) **175**:1983-1990.
83. PORGADOR A, IRVINE KR, IWASAKI A *et al.*: Predominant role for directly transfected dendritic cells in antigen presentation to CD8<sup>+</sup> T cells after gene gun immunization. *J. Exp. Med.* (1998) **188**:1075-1082.
84. CHATTERGOON MA, ROBINSON TM, BOYER JD, WEINER DB: Specific immune induction following DNA-based immunization through *in vivo* transfection and activation of macrophages/antigen-presenting cells. *J. Immunol.* (1998) **160**:5707-5718.
85. ULMER JB, DECK RR, DEWITT CM, DONNHLY JI, LIU MA: Generation of MHC class I-restricted cytotoxic T lymphocytes by expression of a viral protein in muscle cells: antigen presentation by non-muscle cells. *Immunology* (1996) **89**:59-67.

86. FU TM, ULMER JB, CAULFIELD MJ *et al.*: Priming of cytotoxic T lymphocytes by DNA vaccines: requirement for professional antigen presenting cells and evidence for antigen transfer from myocytes. *Mol. Med.* (1997) 3:362-371.
87. WOLFF JA, MALONE RW, WILLIAMS P *et al.*: Direct gene transfer into mouse muscle *in vivo*. *Science* (1990) 247:1465-1468.
88. WOLFF JA, LUDTKE JJ, ACSADI G, WILLIAMS P, JANI A: Long-term persistence of plasmid DNA and foreign gene expression in mouse muscle. *Hum. Mol. Genet.* (1992) 1:363-369.
89. SATO Y, ROMAN M, TIGHE H *et al.*: Immunostimulatory DNA sequences necessary for effective intradermal gene immunization. *Science* (1996) 273:352-354.
90. KLINMAN DM, YI AK, BEAUCAGE SL, CONOVER J, KRIEG AM: CpG motifs present in bacteria DNA rapidly induce lymphocytes to secrete interleukin 6, interleukin 12, and interferon  $\gamma$ . *Proc. Natl. Acad. Sci. USA* (1996) 93:2879-2883.
91. ROSENBERG SA, YANG JC, SHERRY RM *et al.*: Inability to immunize patients with metastatic melanoma using plasmid DNA encoding the gp100 melanoma-melanocyte antigen. *Hum. Gene Ther.* (2003) 14:709-714.
92. TRIOZZI PL, ALDRICH W, ALLEN KO, CARLISLE RR, LOBUGLIO AF, CONRY RM: Phase I study of a plasmid DNA vaccine encoding MART-1 in patients with resected melanoma at risk for relapse. *J. Immunother.* (2005) 28:382-388.
93. PERKUS ME, TARTAGLIA J, PAOLETTI E: Poxvirus-based vaccine candidates for cancer, AIDS, and other infectious diseases. *J. Leukoc. Biol.* (1995) 58:1-13.
94. VAN BAREN N, BONNET MC, DRENO B *et al.*: Tumoral and immunologic response after vaccination of melanoma patients with an ALVAC virus encoding MAGE antigens recognized by T cells. *J. Clin. Oncol.* (2005) 23:9008-9021.
95. LINDSEY KR, GRITZ L, SHERRY R *et al.*: Evaluation of prime/boost regimens using recombinant poxvirus/tyrosinase vaccines for the treatment of patients with metastatic melanoma. *Clin. Cancer Res.* (2006) 12:2526-2537.
96. JAGER E, KARBACH J, GNJATIC S *et al.*: Recombinant vaccinia/fowlpox NY-ESO-1 vaccines induce both humoral and cellular NY-ESO-1-specific immune responses in cancer patients. *Proc. Natl. Acad. Sci. USA* (2006) 103:14453-14458.
97. KAUFMAN HL, CONKRIGHT W, DIVITO J, HORIG H, KALEYA R, LEE D: A Phase I trial of intra-lesional rV-B7.1 vaccine in the treatment of malignant melanoma. *Hum. Gene Ther.* (2000) 11:1065-1082.
98. KAUFMAN HL, DERAFFELE G, DIVITO J, HORIG H, LEE D, PANICALI D: A Phase I trial of intra-lesional rV-TRICOM vaccine in the treatment of malignant melanoma. *Hum. Gene Ther.* (2001) 12:1459-1480.
99. KAUFMAN HL, CHEUNG K, HASKALL Z, HORIG H, HESDORFFER C, PANICALI D: Intra-lesional rF-B7.1 versus rF-TRICOM vaccine in the treatment of metastatic cancer. *Hum. Gene Ther.* (2003) 14:803-827.
100. DUDLEY ME, ROSENBERG SA: Adoptive-cell-transfer therapy for the treatment of patients with cancer. *Nat. Rev. Cancer* (2003) 3:666-675.
101. DUDLEY ME, WUNDERLICH J, NISHIMURA MI *et al.*: Adoptive transfer of cloned melanoma-reactive T lymphocytes for the treatment of patients with metastatic melanoma. *J. Immunother.* (2001) 24:363-373.
102. DUDLEY ME, WUNDERLICH JR, YANG JC *et al.*: A Phase I study of non-myceloablative chemotherapy and adoptive transfer of autologous tumor antigen-specific T lymphocytes in patients with metastatic melanoma. *J. Immunother.* (2002) 25:243-251.
103. ROSENBERG SA, YANNELLI JR, YANG JC *et al.*: Treatment of patients with metastatic melanoma with autologous tumor-infiltrating lymphocytes and interleukin 2. *J. Natl. Cancer Inst.* (1994) 86:1159-1166.
104. ROSENBERG SA, AEBERSOLD P, CORNETTA K *et al.*: Gene transfer into humans: immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. *N. Engl. J. Med.* (1990) 323:570-578.
105. DUDLEY ME, WUNDERLICH JR, ROBBINS PF *et al.*: Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science* (2002) 298:850-854.
- **Seminal article describing a completely innovative and novel approach to immunotherapy via the repopulation of melanoma patients with cloned lymphocytes. This is essential reading for all.**
106. ROSENBERG SA, DUDLEY ME: Cancer regression in patients with metastatic melanoma after the transfer of autologous antitumor lymphocytes. *Proc. Natl. Acad. Sci. USA* (2004) 101:14639-14645.
- **An update of the above article, showing a striking response rate of 51%, which is far above the highest reported rates of any immunotherapy trial to date.**
107. MORGAN RA, DUDLEY ME, WUNDERLICH JR *et al.*: Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science* (2006) 314:126-129.
- **This article highlights the novel use of gene transfer technology and further provides a proof-of-principle that it can be successfully applied to patients with metastatic melanoma.**
108. KLEBANOFF CA, KHONG HT, ANTONY PA, PALMER DC, RESTIFO NP: Sinks, suppressors and antigen presenters: how lymphodepletion enhances T cell-mediated tumor immunotherapy. *Trends Immunol.* (2005) 26:111-117.
109. CURIEL TJ, COUKOS G, ZOU L *et al.*: Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat. Med.* (2004) 10:942-949.
110. SHEVACH EM: Regulatory T cells in autoimmunity. *Annu. Rev. Immunol.* (2000) 18:423-449.
111. JAVIA LR, ROSENBERG SA: CD4<sup>+</sup>CD25<sup>+</sup> suppressor lymphocytes in the circulation of patients immunized against melanoma antigens. *J. Immunother.* (2003) 26:85-93.
112. WOO EY, CHU CS, GOLETZ TJ: Regulatory CD4<sup>+</sup>CD25<sup>+</sup> T-cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. *Cancer Res.* (2002) 61:4766-4772.

113. WOO EY, YEH H, CHU CS: Cutting edge: regulatory T-cells from lung cancer patients directly inhibit autologous T-cell proliferation. *J. Immunol.* (2002) **168**:4272-4276.
114. VIGUIER M, LEMAITRE F, VEROLA O *et al.*: Foxp3 expressing CD4<sup>+</sup>CD25<sup>(high)</sup> regulatory T cells are overrepresented in human metastatic melanoma lymph nodes and inhibit the function of infiltrating T cells. *J. Immunol.* (2004) **173**:1444-1453.
115. SHIMIZU J, YAMAZAKI S, SAKAGUCHI S: Induction of tumor immunity by removing CD25<sup>+</sup>CD4<sup>+</sup> T-cells: A common basis between tumor immunity and autoimmunity. *J. Immunol.* (1999) **163**:5211-5218.
116. GHIRINGHELLI F, LARMONIER N, SCHMITT E *et al.*: CD4<sup>+</sup>CD25<sup>+</sup> regulatory T-cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. *Eur. J. Immunol.* (2004) **34**:336-344.
117. AZUMA T, TAKAHASHI T, KUNISATO A, KITAMURA T, HIRAI H: Human CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells suppress NKT cell functions. *Cancer Res.* (2003) **63**:4516-4520.
118. OLSEN E, DUVIC M, FRANKEL A *et al.*: Pivotal Phase III trial of two dose levels of denileukin diftitox for the treatment of cutaneous T-cell lymphoma. *J. Clin. Oncol.* (2001) **19**:376-388.
119. DANNULL J, SU Z, RIZZIERI D *et al.*: Enhancement of vaccine-mediated anti-tumor immunity in cancer patients after depletion of regulatory T-cells. *J. Clin. Invest.* (2005) **115**:3623-3633.
120. ATTIA P, MAKER AV, HAWORTH LR, ROGERS-FREEZER L, ROSENBERG SA: Inability of a fusion protein of IL-2 and diphtheria toxin (denileukin diftitox, DAB<sub>389</sub>IL-2, ONTAK) to eliminate regulatory T-lymphocytes in patients with melanoma. *J. Immunother.* (2005) **28**:582-592.
121. EISEN T, AHMAD T, FLAHERTY KT *et al.*: Sorafenib in advanced melanoma: a Phase II randomised discontinuation trial analysis. *Br. J. Cancer* (2006) **95**:581-586.
122. FLAHERTY KT, BROSE M, SCHUCHTER L *et al.*: Phase I/II trial of BAY 43-9006, carboplatin (C) and paclitaxel (P) demonstrates preliminary antitumor activity in the expansion cohort of patients with metastatic melanoma. *J. Clin. Oncol.* (2004) **22**:7507-7514.
123. EISEN T, AHMAD T, MARAIS R *et al.*: Phase I trial of sorafenib (BAY 43-9006) combined with dacarbazine (DTIC) in patients with metastatic melanoma. *Eur. J. Cancer Suppl.* (2005) **3**:349-357.
124. KLASA RJ, GILLUM AM, KLEM RE *et al.*: Oblimersen Bcl-2 antisense: facilitating apoptosis in anticancer treatment. *Antisense Nucleic Acid Drug Dev.* (2002) **12**:193-213.
125. BEDIKIAN AY, MILLWARD MJ, PEHAMBERGER H *et al.*: Bcl-2 antisense (oblimersen sodium) plus dacarbazine in patients with advanced melanoma: the Oblimersen Melanoma Study Group. *J. Clin. Oncol.* (2006) **24**:4738-4745.
126. RIBAS A, CAMACHO LH, LOPEZ-BERESTEIN G *et al.*: Antitumor activity in melanoma and anti-self responses in a Phase I trial with the anti-cytotoxic T lymphocyte-associated antigen 4 monoclonal antibody CP-675,206. *J. Clin. Oncol.* (2005) **23**:8968-8977.
127. HERSH EM, WEBER J, POWDERLY J *et al.*: A Phase II, randomized multi-center study of MDX-010 alone or in combination with dacarbazine (DTIC) in stage IV metastatic malignant melanoma. *J. Clin. Oncol. (Post-Meeting Edition)* (2004) **22**:14S.
128. MAKER AV, PHAN GQ, ATTIA P *et al.*: Tumor regression and autoimmunity in patients treated with cytotoxic T-lymphocyte-associated antigen 4 blockade and interleukin-2: a Phase I/II study. *Ann. Surg. Oncol.* (2005) **12**:1005-1016.
129. MAKER AV, ATTIA P, ROSENBERG SA: Analysis of the cellular mechanism of antitumor responses and autoimmunity in patients treated with CTLA-4 blockade. *J. Immunol.* (2005) **175**:7746-7754.
130. ATTIA P, PHAN GQ, MAKER AV *et al.*: Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. *J. Clin. Oncol.* (2005) **23**:6043-6053.
131. PHAN GQ, YANG JC, SHERRY RM *et al.*: Cancer regression and autoimmunity induced by cytotoxic T-lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. *Proc. Natl. Acad. Sci. USA* (2003) **100**:8372-8377.
132. GOTHELF A, MIR LM, GEHL J: Electrochemotherapy: results of cancer treatment using enhanced delivery of bleomycin by electroporation. *Cancer Treat. Rev.* (2003) **29**:371-387.
133. SERSA G, CEMAZAR M, MIKLAVCIC D: Antitumor effectiveness of electrochemotherapy with cis-diamminedichloroplatinum (II) in mice. *Cancer Res.* (1995) **55**:3450-3455.
134. ROLS MP, DELTEIL C, GOLZIO M, DUMOND P, CROS S, TEISSIE J: *In vivo* electrically mediated protein and gene transfer in murine melanoma. *Nat. Biotechnol.* (1998) **16**:168-171.
135. GEHL J, SORENSEN TH, NIELSEN K *et al.*: *In vivo* electroporation of skeletal muscle: threshold, efficacy and relation to electric field distribution. *Biochim. Biophys. Acta* (1999) **1428**:233-240.
136. ENGSTROM PE, PERSSON BR, SALFORD LG: Studies of *in vivo* electroporation of (99m)Tc-DTPA. *Biochim. Biophys. Acta* (1999) **1473**:321-328.
137. ROLS MP, BACHAUD JM, GIRAUD P, CHEVREAU C, ROCHE H, TEISSIE J: Electrochemotherapy of cutaneous metastases in malignant melanoma. *Melanoma Res.* (2000) **10**:468-474.
138. SERSA G, STABUC B, CEMAZAR M, MIKLAVCIC D, RUDOLF Z: Electrochemotherapy with cisplatin: clinical experience in malignant melanoma patients. *Clin. Cancer Res.* (2000) **6**:863-867.
139. MIR LM, GLASS LF, SERSA G *et al.*: Effective treatment of cutaneous and subcutaneous malignant tumors by electrochemotherapy. *Br. J. Cancer* (1998) **77**(12):2336-2342.
140. HELLER R, JAROSZESKI MJ, GLASS LF *et al.*: Phase I/II trial for the treatment of cutaneous and subcutaneous tumors using electrochemotherapy. *Cancer* (1996) **77**(5):964-971.
141. RODRIGUEZ-CUEVAS S, BARROSO-BRAVO S, ALMANZA-ESTRADA J, CRISTOBAL-MARTINEZ L, GONZALEZ-RODRIGUEZ E: Electrochemotherapy in primary and metastatic skin tumors: Phase II trial using intralesional bleomycin. *Arch. Med. Res.* (2001) **32**(4):273-276.

142. HELLER L, MERKLER K, WESTOVER J *et al.*: Evaluation of toxicity following electrically mediated interleukin-12 gene delivery in B-16 mouse melanoma model. *Clin. Cancer Res.* (2006) **12**(10):3177-3183.
143. LI S, ZHANG X, XIA X: Regression of tumor growth and induction of long-term antitumor memory by interleukin-12 electrogene therapy. *J. Natl. Cancer Inst.* (2002) **94**(10):762-768.
144. BYRNE CM, THOMPSON JF: Role of electrochemotherapy in the treatment of metastatic melanoma and other metastatic and primary skin tumors. *Expert Rev. Anticancer Ther.* (2006) **6**:671-678.
145. MORTON DL, THOMPSON JF, COCHRAN AJ *et al.*: Sentinel-node biopsy or nodal observation in melanoma. *N. Engl. J. Med.* (2006) **355**(13):1307-1317.
- **Important article that reports on the results of the MSLT-I trial and further showing that it is beneficial for patients to undergo a sentinel node mapping procedure as opposed to wait-and-watch approach.**
146. TAGAWA ST, CHEUNG E, BANTA W, GEE C, WEBER JS: Survival analysis after resection of metastatic disease followed by peptide vaccines in patients with Stage IV melanoma. *Cancer* (2006) **106**(6):1353-1357.
147. YANG JC, ABAD J, SHERRY R: Treatment of oligometastases after successful immunotherapy. *Semin. Radiat. Oncol.* (2006) **16**(2):131-135.

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