Chapter 1

Advances of Immunotherapy in Melanoma: Where do we stand?

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Background

Malignant melanoma, well known as the deadliest type of skin cancer, registered an exponential increase in incidence in the last decade [1]. According to ESMO Clinical Practice Guidelines, Europe reports a variation from 3 to 5 new cases of cutaneous melanoma/100 000/year in Mediterranean countries and 12 to 25/100 000/year in northern Europe [2]. Despite the numerous therapeutic strategies, malignant melanoma remains the most aggressive type of cancer due to its unpredictable evolution [3]. Late-stages melanomas have a rapid capacity of development and metastasis, frequently gaining therapeutic resistance and develop recurrence, with a median survival rate less than 12 months. For this reason, prevention and early diagnosis of melanoma have been highly promoted in the last years.

The mechanisms involved in melanomagenesis and the sequence of the events leading to aggressive forms of melanoma are now being discovered. A state of "chronic inflammation" governs the tumor microenvironment, responsible for many mechanisms such as resistance to apoptosis, adaptive responses, proliferation and angiogenesis. However, these intriguing pathways that start up with the modulation of the immune system within the tumor and continue with the proliferation and metastasis to distant sites of the body are not yet elucidated.

Tumor promoting inflammation along with avoidance of immune destruction, enables replicative immortality, escape from growth suppressors, sustained prolifer-

ative signaling, deregulated cellular energetics, resistance to cell death, genome instability and mutation, angiogenesis invasion and metastasis, the so called, "hallmarks of cancer" [4].

Moreover, most of the commonly used cancer therapies like chemotherapy, ionizing radiation and major surgery are immunosuppressive. The ideal cancer therapy should not only destroy the primary tumor, but at the same time trigger the immune system to recognize, track down and destroy any remaining tumor cells that are at or near the site of the primary tumor or distant micro metastases. Melanoma is one of the most immunogenic tumor types mainly because many lymphocytes responsible with the development of this type of cancer are an important component of tumor milieu [5]. In addition, the metastatic disease seems to respond to immune stimulatory agents [6]. Melanoma immunotherapy has gained popularity in oncology by starting with the theory that the body's own weapons, the immune cells, can be activated to fight against cancer. This includes many strategies such as therapeutic cancer vaccines, cytokine administration and immune cell–based therapies. In addition, there is an increased interest in understanding T-cells and NK-cells regulatory pathways that conducted to the study of a novel class of immune checkpoint inhibitors.

These immunotherapeutic weapons against melanoma include interferon alpha-2 beta (IFN α-2β), interleukin-2 and the newest developed blocking antibodies anti-cytotoxic Tlymphocyte associated antigen-4 (CTLA-4) and programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1).

A strong parallel between the cancer and pregnancy has been proposed over the recent years. As a single malignant cell could proliferate and then metastasize undetected within a host similarly a semiallogenic fetus escapes from the maternal immune system. In addition, a resemblance between the immune cells from the tumor microenvironment that keep the cancer cells untouched by the host's attack mechanisms and the feto-maternal interface that is constituted by the placenta that induces a plethora of immunoregulatory properties. Regulatory T cells (Treg's) play an important role in the orchestration of these regulatory mechanisms both in cancer and pregnancy [7]. A decrease of this specific lymphocyte population is associated with eclampsia and abortion. Expanding this notion, in oncology studies the suppression of Treg's raised the antitumor immune system and improving the prognosis of the patients. Understanding the T regulatory cells mechanisms lead to the development of the immune check-point inhibitors that are a promising ally in the fight against cancer, particularly the case of melanoma. Thus, these recent advances in immunotherapy (CTLA-4 and

PD1/PDL-1 inhibitors) altered the dramatic outlook of the patients with advanced melanoma that previously had few therapeutic options. Still, these drugs remain under intense investigation due to their important side effects.

Although there are not the subjects of our debate in this chapter, a brief mention of the kinase inhibitors should be mentioned, as they stand as the corner stone of melanoma therapy. Progressive multiple genetic alterations seem to be responsible for the transformation of benign melanocytic naevi to melanoma lesions. A major discovery in the management of melanoma was the oncogenic mutation in the serine threonine kinase BRAF [8]. Studies showed that position V600E BRAF mutations are harbored by more than a half of all cutaneous melanoma patients, which involves the RAS-RAF-MEK-ERK pathway activation. This is important for cell survival, proliferation and resistance to apoptosis [9-10]. Knowing this, a plethora of kinase inhibitors were developed as new therapeutic strategies against malignant melanoma [11]. For the treatment of systemic metastatic melanoma (stage IV) were synthesized selective BRAF inhibitors, such as Vemurafenib, Dabrafenib, Encorafenib, which can be administered alone or combined with MEK inhibitors, Binimetinib, Trametinib, Cobimetinib (Figure 1 and Figure 2) [2].

Randomized studies showed that BRAF inhibition improve progression-free and overall survival in metastatic malignant melanoma, compared to the gold-standard chemotherapeutic drug Dacarbazine [12]. The association of BRAF and MEK inhibitors demonstrated to be more powerful compared to the BRAF inhibition alone [13,14].

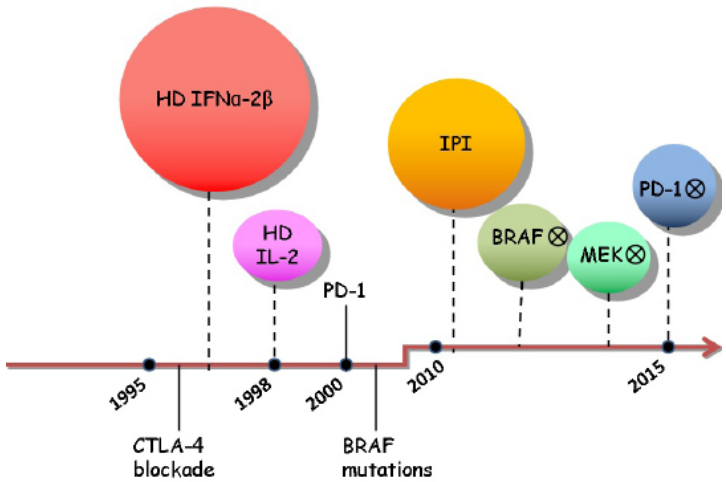

Figure 1: Discovery of the immune therapies and BRAF/MEK pathway inhibitors, corner stoners in the treatment of advanced melanoma.

Even though BRAF/MEK inhibitor doublet improved the rate of progression-free survival, delayed the develop-

ment of resistance and registered reduced toxic side effects in patients with BRAF mutations, the long-term responsiveness is still limited by the adaptive drug resistance [15]. Therefore, other personalized combination strategies try to improve the response durability.

For instance, the combination of Nivolumab and Ipilimumab showed rapid and deep responses in 80% of advanced melanoma patients, after 12 weeks treatment [16]. Moreover, it seems that BRAF inhibitors induce higher objective response rate than those treated with chemotherapy [17]. Another recent study showed that NK-cell based immunotherapy (low dose of IL-2) combined with a BRAF inhibitor, Vemurafenib can promote durable responses [18]. Moreover, a new clinical trial that is currently under investigation assesses the efficacy of the combination Vemurafenib and IFN alpha-2b (Clinical Trials.gov identifier NCT01943422). ESMO Clinical Practice Guidelines consider kinase inhibitors and immunotherapy the backbone of cutaneous melanoma systemic therapy [2].

This chapter reviews the current strategies of immunotherapy for melanoma, highlighting the new advances in this domain.

Tumor Immune Survey *Awakening*

Melanoma is a highly immunogenic tumor [19]. Melanoma cells express tumor-specific antigens, which

can be presented to CD8+ lymphocytic T cells along with major histocompability complexes class I (MHC-1) and to CD4+ T lymphocytes with MHC class II on the macrophages or other antigen-presenting cells (APC). Several antigens were identified in both normal melanocytes and melanoma cells, like: MelanA/MART-I, MAGE-1, gp100, tyrosinase, and tyrosinase-related proteins [20]. Morphopatology examination of melanoma spontaneous regression areas, showed infiltrates with T lymphocytes and melanophages [21,22].

Interferon and IL-2

Firstly observed by Isaacs and Lindenmann, afterwards approved by the U.S. Food and Drug Administration (FDA)in 1995, Interferon alfa-2 beta(IFNα-2β) is the first immunotherapeutic agent which showed significant results as monotherapy in patients with melanoma at hight risk of recurrence [23,24]. IFNα-2β is well known for its indirect immunoregulatory effects, antiangiogenic, antiproliferative, proapoptotic and differentiation-stimulation [25].

Different trials evaluated whether low dose, intermediate dose or high dose of IFNα-2β has a greater beneficial impact. An optimal treatment scheme regarding dosage, frequency, duration, administration route and management of side effects is still missing, fact that raised many issues and limited the clinical use [24].

Significant data provided by 2 meta-analyses conducted by Wheatley et al. (13 randomized trials involved) and Mocellin et al. (14 randomized trials involved) showed an absolute statistically impact on both overall-survival and disease-free survival, regardless of dose and length of treatment [26,27].

Paolo A. et al. explain the uncertainty regarding the IFNα-2β dose and duration of therapy as a consequence of differences in responsiveness in patients from distinct populations to immunotherapy [24]. Even though meta-analyses do not support the theory that only high-dose IFN might bring the best OS benefit, ESMO Clinical Practice Guidelines recommend high-doses of IFNα-2β for high-risk surgically resected melanoma [28].

According to disease stage, the Italian expert panel make the following recommendations: stage IIA - no treatment, stage IIB – low doses IFN(for 18-24 months), stage IIC – low/high doses IFN, stage IIIA-IIIB - low/high dose IFN or Peg IFNα-2β, stage IIIC – high dose IFN [24]. However, DeCOG study showed that low-doses of IFN administered for 24 months induced an important benefit on both disease-free and overall survival, especially when sentinel lymph node biopsy was positive for metastases [29]. Moreover, the same German Group proved that prolonged low-dose IFN administration (60 months) to patients with Breslow index≥1.5 mm does not improve the

overall survival or disease-free survival, compared with standard treatment duration (18 months) [30].

Despite intense research, the precise mechanism of action of IFNα-2β and the dose-dependent-differential effect could not be established. However, main evidence suggests that high-dose IFNα-2β acts as an indirect immunomodulator via tumor cells infiltration, increase in STAT1/STAT3 ratio in tumor cells, development of autoantibodies, circulating Treg cells reduction and changes in cytokines serum levels, all these being responsible for increased anti-tumor response [31-34].

Serum cytokines, growth and angiogenic factors levels were observed comparatively in healthy individuals and in patients treated with high-dose IFNα-2β included in the ECOG1694 trial [35,36].Surprisingly, high-dose IFN treated patients with RFS over 5 years showed higher pretreatment cytokines levels(TNF-α, IL-1, IL-6) than in patients with early recurrence. Treated patients and healthy individuals had an increased serum level of: IFNα, IP-10, TNFR-I, TNFR-II, MCP-1, IL-2R and a decreased level of VEGF, EGF, HGF. Therefore, initial serum levels of some cytokines might be a useful predictor of high-dose IFN treatment responsiveness.

Pegylated IFNα-2β (Peg IFNα-2β) appeared in 2011 as an enhanced form of IFNα-2β. Being able to maintain a prolonged plasma concentration (decreased clearance and

increased half-life) than IFNα-2β, Peg IFNα-2β allows one administration/week with greater efficacy. Treatment with Peg IFNα-2β showed a significant improvement in recurrence-free survival, but unfortunately no improvement in overall survival. In 2013, the EADO randomized study compared lower doses of Peg- IFNα-2β (36 months) with lower doses of IFNα-2β(18 months), showing no significant difference in overall survival and disease free survival [37]. Besides, these data suggests that even though Peg IFNα-2β is efficacious as an adjuvant treatment of melanoma, its superiority over IFNα-2β has not been demonstrated. Moreover, it is difficult to sustain a treatment with Peg IFNα-2β for several years due to its adverse effects (headache, depression, fatigue, pyrexia, hepatotoxicity and myalgia), the median administration duration being 19.2 months [38]. Clinically benefit was obtained when patients with macrometastatic melanoma received intravenous high-doses IFNα-2β, while those with ulceration of primary lesion and micrometastases responded to lower-doses IFNα-2β or Peg IFNα-2β. This means that IFN treatment efficacy differs depending on whether micro or macroscopic disease is involved. EORTC 18952 and EORTC 18991 studies proved that melanoma stage, tumor burden and ulceration of primary melanoma represent predictive factors, being correlated with IFN response in melanoma patients [39].

Adjuvant therapy with cytokines like IL-2 was intense studied since 1985 for late-stages melanoma. Even though high doses of IL-2 induce cytolysis at the tumor site via T cells and NK cells, with a greater potential than IFNα-2β, its severe acute toxicity limit the systemic IL-2 administration (arrhythmias, hypotension, hepatotoxicity, severe weight gain, anaemia, leucocytopaenia and thrombocytopenia, vascular leak syndrome) [40]. Therefore, patients must have a relatively normal pulmonary, heart and kidney status previous to IL-2 administration.

Intralesional IL-2 regimens produced an increased local beneficial effect with reduced systemic side effects when used for patients with cutaneous melanoma metastases [41,42]. Latest ESMO guidelines recommend that adjuvant therapy with IL-2 should not be used outside controlled clinical trials [28]. Recent studies wanted to explore which is the maximally beneficial dose with the most reduced side effects. Thus, it seems that the higher-dose and more frequently administerd, the most durable and beneficial responses associated with higher-grade toxicity. Currently, new combinations of high dose IL-2 with anti-CTLA4 antibody, as well as IFNα-2β therapy are being investigated as a curable treatment regimen for late stages melanoma [43]. Unfortunately, this combined strategy did not bring so far a real therapeutic improvement [44,45].

Photodynamic Therapy

Photodynamic therapy (PDT) has been approved in many countries for the treatment of lung, esophageal, bladder, skin head and neck cancers. It is a minimally invasive two-stage procedure that requires prior administration of a photosensitizing agent (PS) followed by light activation. This leads to subsequent biochemical events that cause a combination of direct tumor cell photodamage, destruction of tumor vasculature and activation of an immune response [46,47].

PDT produces an acute inflammatory response that is considered important for the activation of antitumor immunity [46]. The acute inflammation induced by PDT characterized by leucocytes infiltration, predominantly neutrophils into the tumor is likely to be caused by the enhanced expression of two transcription factors, nuclear factor kappa B (NFκB) and activator protein 1 (AP1) [48]. Serum increase of cytokines and chemokines, such as: IL6, macrophage inflammatory protein 1 (MIP1) and MIP2 were reported in mice following PDT directed to a subcutaneous tumor or even to normal skin. Increased levels of IL1β, IL6, IL8 and IL10 were detected in patients after surgery and intra-operative thorax PDT for mesothelioma using Foscan 0.1 mg/kg, 6 days before surgery and 10 J/cm2 (652 nm) [49].

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PDT also induces the expression of stress proteins like: heat shock proteins HSP70 [50], HSP47 [48], HSP60 [51] and stress-inducible proteins: glucose-regulated protein 78 (GRP78) [52], GRP94 [53] and heme oxygenase [54].

In fact, PDT induced in the treated area of the tumor the generation of "danger" signals, so called damage-associated molecular patterns (DAMPs), which are intracellular molecules released as "warning signals" from dying and/or damaged cells [55]. It seems that DAMPs are key mediators that inform the immune system about releasing of "self-altered antigens" and trigger a specific immunological response. DAMPs are released from killed tumor cells via necrosis or apoptosis and activate immune cells like macrophages, certain T cells, NK cells, and dendritic cells (DCs) [56]. In PDT, the best known DAMPs include heat-shock protein (HSP) family (HSP70 and HSP90), high mobility group box-1 (HMGB-1), adenosine triphosphate (ATP), and calreticulin (CRT). The release of HSP-bound tumor antigens from necrotic tumor cells further activates phagocytosis and presentation by antigen presenting cells (APC) and increases the antigen cross-presentation to efficiently stimulate the formation of tumor-specific cytotoxic T lymphocytes. Thus, pro-inflammatory effects of PDT might increase dendritic-cell migration, antigen uptake and maturation (Figure 2) [46].

It is generally accepted that only necrotic cells are immunogenic [57] while the apoptosis is not able to trigger an immune response except for certain cancer therapies which can induce so called "immunogenic apoptosis" [58]. Korbelik et al described for the first time that squamous cell carcinoma cells, treated by photofrin-PDT, exposed on the surface heat shock proteins (HSPs) and glucose-regulated protein 94 (GRP94) [59]. In the same cancer cells but on an in vivo model, they found other different proteins from DAMPs group exposed on the cells surface. This suggested that in PDT, DAMPs exposed and/ or released are correlated with the type of photosensitizer (PS) and its sub-cellular localization. Hypericin targets the endoplasmatic reticulum and consequently exposes calreticulin (CRT) at the cell surface while Photofrin, whose localization is associated with lipid membranes, exposes HSP70 [60]. Moreover, the oxidative stress generated at specific subcellular sites, through the light activation of organelle-associated PS, is involved in DAMPs induction [61].

HSP70 forms stable complexes with different tumor antigens which, after binding to Toll-like receptors 2 and 4, on the surface of dendritic cells induce transition to the their mature state. Dendritic cells express peptide-MHC complexes on their surface and after recognition by CD4+ T helper cells and CD8+ cytotoxic T lymphocytes, they are able to initiate an adaptive immune response. In addition, HSP70 stimulates the activation of NK cells [62].

The growth inhibition of murine EMT6 tumors after Photofrin-PDT was dependent on the presence of CD8+ T cells. CD8+ and CD4+ T cells from mice that survived cancer were transferred three months after vascular targeted PDT with bacteriochlorophyll derivative WST11. The receiving mice were protected from subsequent challenge with viable cancer cells.

Besides T lymphocytes, B cells produce antigen-specific immunoglobulins and are responsible for the humoral immune response in PDT [63]. Although there are ample evidences regarding the involvement of humoral immunity in PDT, the role of the humoral components in tumor destruction is not fully cleared and needs further investigations. PDT may also interfere with immune-suppressive T cells especially CD4+CD25+FoxP3+ T regulatory cells (Treg) [64].

Several studies reported enhanced immune-stimulatory effects after **PDT combined with other immune therapies** like local tumor administration of dendritic cells, microbial adjuvants and/ or systemic IFN α-2β.

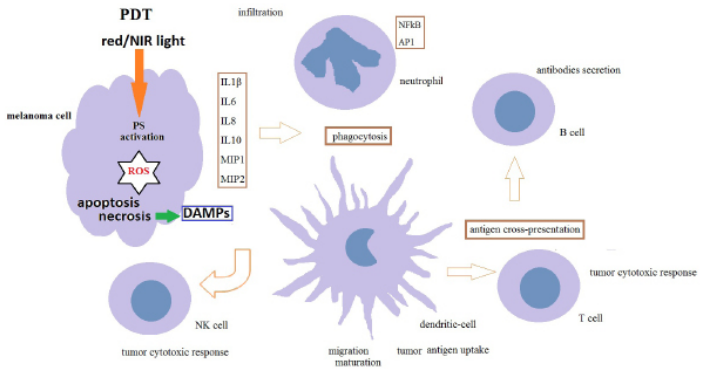

Figure 2: Photodynamic therapy effects on local immunity response.

Photosensitiser (PS) activation by light of the correct wavelength creates oxygen free radicals (ROS) responsible for the killing of the tumor cell by apoptosis and/or necrosis. PDT damaged cells, mainly necrotic cells lead to the exposure on the cell surface of the damage-associated molecular patterns (DAMPs), proteins from the group of heat shock proteins (HSP), high mobility group box-1 (HMGB-1), adenosine triphosphate (ATP), and calreticulin (CRT). These proteins function as chemotactic signals for neutrophyls and macrophages that infiltrate the tumor, maturate and stimulates phagocytosis, processes mediated by activation of the transcription factors NFkB (nuclear transcription factor kB) and AP1 (activator protein 1). PDT is also followed by local and serum increase of

cytokines and chemokines, such as: IL1β, IL6, IL8, IL10, macrophage inflammatory protein 1 (MIP1) and MIP2 with roles in the activation of the immune response. Dendritic cells increase the antigen uptake, presentation and chemokine secretion, which in turn triggers a T cytotoxic and B antibody secretion response to the tumor antigens.

PDT was reported to induce cures and long-lasting tumor-specific immunity (memory), as shown by the rejection of tumors on rechallenge in several mouse and rat models. PDT and intra-tumor injection of naïve dendritic cells (DC) significantly eradicated CT26 colorectal carcinoma cells and B16 melanoma in BALB/c or C57BL6 mice or prolonged the survival of the animals. Moreover, PDT plus naïve DC administered at a tumor site led to distant tumors regression, including multiple lung metastases. However, PDT or intra-tumor administration of naïve DC as a single treatment was ineffective [65]. PDT affects monocyte/macrophage and lymphocyte cell lineages.

Lymphocytes are also killed by PDT, as shown in a report using DBA/2 mouse thymocytes treated with verteporfin PDT, and activated human T lymphocytes are especially susceptibl [66]. T-regulatory cells might be specifically inactivated by IL6 [67].

Association of PDT with intralesional γ -inulin (0.1 mg/mouse), activated the complement similarly to zymosan and delayed the recurrence of B16-BL6 melanomas in mice. This effect was further enhanced by IFN pre-

treatment [68]. IFN α-2β might enhance the expression of MHC on APC, which makes tumor antigen presentation to lymphocytic cells more efficient. Patients treated with IFN showed an increased lymphocytic infiltration of the melanoma lesions [69]. PDT or γ –inulin administration increased the C3 level in the tumor. C3 protein was further increased when a combination therapy was used. Thus, at 3 days after the treatment with PDT plus γ-inulin, more than half of the cells found at the tumor site were cytotoxic T lymphocytes engaged in killing specific targets via the perforin–granzyme pathway [67].

Cancer vaccines preparation using PDT treated cell cultures in vitro were attempted. The PDT treated cell lysates administered to tumor bearing mice, induced phenotypic DC maturation and IL12 expression, and produced tumor regression, growth retardation and even some cures [70].

The vaccine cells retrieved at 1 hour from the treatment site were mixed with DC, expressed on their surface HSP70 and were opsonized by complement C3 [71].

PDT was shown to suppress contact hypersensitivity reaction in mice but not delayed type hypersensitivity, involved in anti-tumor immunity [46,71].

Immune Check-Point Inhibitors

Tumor cells display many similarities in their progression of conquering the host. In 2000 Hannahan and Weinberg have reunited these common traits, also known as the "hallmarks of cancer", as following: 1) Self-sufficiency in growth signals, 2) Insensitivity to anti-growth signals, 3) Evading apoptosis, 4) Limitless replicative potential, 5) Sustained angiogenesis, 6) Tissue invasion and metastasis [72]. Later they added other four properties: deregulated metabolism, unstable DNA, inflammation and immune system escape [4].

Cancer development implies the presence of many immune suppressive and immune evasion mechanisms. The concept of immunotherapy developed in oncology in order to target the cancer cells with the host's own immune cells. A debated subject in the immunology of cancer is the T regulatory lymphocytes (Treg's). Treg's are infiltrated along with other immune cells in the tumor microenvironment and induce host immunity suppression through numerous pathologic mechanisms [73]. Understanding the Treg's biology led to the development of the cornerstone of immunotherapy: the check-point inhibitors. Tumor cells use CTL antigen-4 (CTLA-4) and programmed death-1 (PD-1) inhibitory pathways in order to decrease the host's immune cells activity. An in vivo study on mice bearing B16 melanoma revealed that regulatory T tumor lymphocytes expressed PD-1, CTLA-4 or both markers

[74]. Therefore, blocking of PD-1 and CTLA-4 can modulate Treg functions, leading to antitumor responses and tumor rejection (Figure 3).

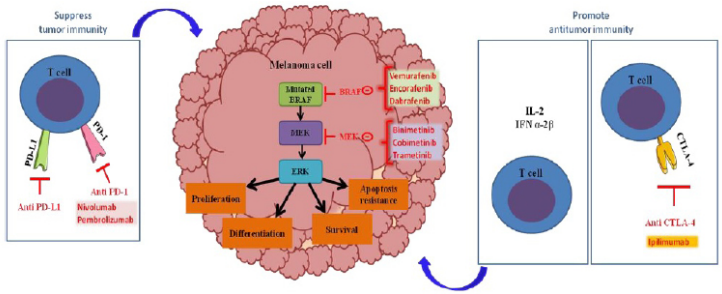

Figure 3: Therapeutic targets in melanoma. Tumor cells that have a BRAF mutation display a defective BRAF-ERK pathway activation that switch the normal regulation of cell proliferation/differentiation to apoptosis resistance and increased survival; the action sites for BRAF and MEK inhibitors are presented. Immunotherapy in melanoma is based on promoting the T cell mediated antitumor immunity (right panel) by the use of IFNα-2β and IL-2 and Ipilimumab (antibody against CTLA-4 surface receptor) and suppression of the tumor inhibition of host immunity response, mediated by Treg's, consisting of antibodies against PD-1/PD-L1 (left panel).

Following these assumptions, an important phase III randomized clinical trial compared Ipilimumab with glycoprotein 100 vaccine (gp100) and reported a new outbreak in oncology [75]. Among the 676 patients enrolled in the clinical trial, 403 received Ipilimumab associated with gp100, 137 received Ipilimumab alone, and the last group of 136 patients received only gp100. Ipilimumab increased the median overall survival with 10.0 months, whether administered alone or in combination, compared with 6.4 months in the peptide alone arm (hazard ratio, 0.68; $P < 0.003$). The drug Ipilimumab also demonstrated its effectiveness in another clinical trial compared with Dacarbazine, with a total duration of the therapeutic outcome of 19.3 months for the Ipilimumab arm versus 8.1 months for Dacarbazine [76]. Thus, in the early 2011, FDA authorized the use of Ipilimumab (dose of 3 mg/kg IV every 3 weeks for 4 cycles of treatment, without maintenance). A second CTLA-4–blocking agent, Tremelimumab, did not demonstrate an improvement in overall survival compared with standard chemotherapy [77].

The programmed death 1 (PD-1) receptor, expressed on antigen-stimulated T cells is a negative regulator of the T lymphocytes, silencing their antitumor actions. PD-1 bounds to its ligands: programmed cell death-ligand 1(PD-L1) and PD-L2 (also known as B7-H1 and B7-DC) and leads to defective effector T-cells: inhibition of cytotoxicity, cytokine production and proliferation. Physiologically, this receptor maintains the peripheral immune tolerance and limits excessive tissue damage during

acute inflammation. In the context of chronic inflammation and cancer, an elevated PD-1 level was found responsible for a state of T-cell "exhaustion", and therefore a decreased immune response. In 2012, the anti–PD-1 antibody Pembrolizumab was tested in phase I trials in patients with advanced melanoma, including those with disease progression while they had been receiving Ipilimumab. Surprisingly, eighty-seven percent of patients had durable responses ranging from more than 2.6 months to more than10 months. Following Pembrolizumab administration a high rate of tumor regression was registered. Therefore, FDA approved Pembrolizumab as second- or third-line therapy in patients with prior Ipilimumab treatment and BRAF V600 mutant with prior BRAF-directed therapy. Another targeted PD-1 antibody tested in clinical trials is Nivolumab [78]. Nivolumab demonstrated an improved activity in patients with the wild type BRAF melanoma [79]. Other anti PDL-1 antibodies (BMS-936559 and MPDL-3280A) proved an important therapeutic response in early clinical trials [80,81].

Because these targeted immune check-point inhibitors revealed great success in clinical trials, oncologists thought of the association with other therapies to increase the efficacy. A plethora of trials then emerged: the association of PDL-1 antibodies with radiotherapy, BRAF/MEK inhibitors or immune modulatory agents. Also, a concomitant administration of anti-CTLA-4 and anti- PD-1

antibodies revealed a greater response compared with their administration alone, although with higher adverse effects.

Radiotherapy induced in some cases an abscopal effect, a phenomenon where not only the primary tumor was shrinking after the localized treatment, but also the distant metastases. This phenomenon was described in a series of case reports when palliative radiotherapy was associated after the administration of Ipilimumab due to the disease regression [82]. An efficient antitumor response was reported. The combination of checkpoint blockade and BRAF inhibitors was previewed as a great success, as each therapy is a cornerstone in the management of metastatic melanoma. However, initial trials revealed dose-limiting hepatic toxicities and needed to be stopped [83]. A decrease of toxicity and an increased response was observed when Ipilimumab was associated with the granulocyte-macrophage colony-stimulating factor vaccine (GM-CSF), Sargramostim.

The combination of the anti-CTLA4 antibody and the vaccine revealed an improved overall survival compared with Ipilimumab alone (median OS 17.5 vs. 12.7 months, 1-year OS, 68.9% vs. 52.9%, $p = 0.01$ for both comparisons) as well as lower toxicity in the combination cohort (grade 3–5 adverse events in 44.9% vs. 58.3%, $p = 0.04$) [84].

Although the check-point inhibitors have been a revolution in oncology in the past years, clinicians must have an important background in understanding the biology of these antibodies and their adverse reactions. With close patient monitoring the adverse effects are usually reversible. These include: skin-related adverse events like rash and pruritus, liver and gastrointestinal effects, endocrinopathies and general symptoms like nausea, fever, and fatigue [85-87].

The most important combination regimen was concomitent CTLA-4 and PD-1 blockade that showed a high potential for rapid and good responses, although with increased toxicity. In a phase I study, patients were treated concurrently with Ipilimumab and Nivolumab and an objective response rate of 40% was seen across all cohorts. The maximum administered doses were 3 mg/kg of Ipilimumab and 1 mg/kg of Nivolumab [88]. Although an important therapeutic response was observed, the mechanisms of immune check-point inhibitors remain to be discovered. It is the beginning of a new era in oncology, of individual therapy, where our own forces (the immune cells) can fight the cancerous cells.

Conclusions

Melanoma treatment seems to be an enormous chest, filled with boxes containing anti-cancer strategies, in which one can discover smaller boxes with specific targeted anti-cancer molecules and other anti-neoplastic agents. As no one knows which box might reveal the most effective cure for melanoma, scientists gradually open new ones and discover new key-molecules, going closer and closer to the answer. Thus, the near future might be brighter than we expected for the patients suffering from melanoma.

References

1. Merrill SJ, Ashrafi S, Subramanian M, Godar DE. Exponentially increasing incidences of cutaneous malignant melanoma in Europe correlate with low personal annual UV doses and suggests 2 major risk factors. Dermato-Endocrinology. 2015; 7: e1004018.
2. Dummer R, Hauschild A, Lindenblatt N, Pentheroudakis G, Keilholz U; ESMO Guidelines Committee. Cutaneous melanoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2015; 26: v126-132.
3. Abdel-Rahman O, ElHalawani H, Ahmed H. Doublet BRAF/MEK inhibition versus single-agent BRAF inhibition in the management of BRAF-mutant advanced melanoma, biological rationale and meta-analysis of published data. Clinical and Translational Oncology. 2015: 1-1.
4. Hanahan D, Weinberg RA. Hallmarks of cancer:

the next generation. Cell. 2011; 144: 646-674.

5. Boon T, Coulie PG, Van den Eynde BJ, van der Bruggen P. Human T cell responses against melanoma. Annu Rev Immunol. 2006; 24: 175-208.
6. Garbe C, Eigentler TK, Keilholz U, Hauschild A, Kirkwood JM. Systematic review of medical treatment in melanoma: current status and future prospects. Oncologist. 2011; 16: 5-24.
7. Holtan SG, Creedon DJ, Haluska P, Markovic SN. Cancer and pregnancy: parallels in growth, invasion, and immune modulation and implications for cancer therapeutic agents. Mayo Clin Proc. 2009; 84: 985-1000.
8. Davies H, Bignell GR, Cox C, Stephens P, Edkins S. Mutations of the BRAF gene in human cancer. Nature. 2002; 417: 949-954.
9. Sosman JA, Kim KB, Schuchter L, Gonzalez R, Pavlick AC. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. N Engl J Med. 2012; 366: 707-714.
10. Dhomen N, Marais R. BRAF signaling and targeted therapies in melanoma. Hematol Oncol Clin North Am. 2009; 23: 529-545, ix.
11. Fedorenko IV, Gibney GT, Sondak VK, Smalley KS. Beyond BRAF: where next for melanoma therapy? Br J Cancer. 2015; 112: 217-226.
12. da Rocha Dias S, Salmonson T, van Zwieten-Boot B, Jonsson B, Marchetti S, et al. The European Medicines Agency review of vemurafenib (ZelborafÂ®) for the treatment of adult patients with BRAF V600 mutation-positive unresectable or metastatic melanoma: summary of the scientific assessment of the Committee for Medicinal Products for Human Use. European Journal of Cancer. 2013; 49: 1654-1661.
13. Long GV, Stroyakovskiy D, Gogas H, Levchenko E, de Braud F. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. N Engl J Med. 2014; 371: 1877-1888.
14. Gibney GT, Atkins MB. Immunotherapy or molecularly targeted therapy: what is the best initial treatment for stage IV BRAF-mutant melanoma. Clinical advances in hematology & oncology: H&O. 2015; 13: 451-458.
15. Holderfield M, Deuker MM, McCormick F, McMahon M. Targeting RAF kinases for cancer therapy: BRAF-mutated melanoma and beyond. Nat Rev Cancer. 2014; 14: 455-467.
16. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013; 369: 122-

Recent Advances in Skin Cancer

133.

17. Margolin K, Ernstoff MS, Hamid O, Lawrence D, McDermott D. Ipilimumab in patients with melanoma and brain metastases: an open-label, phase 2 trial. Lancet Oncol. 2012; 13: 459-465.

18. Ferrari de Andrade L, Ngiow SF, Stannard K, Rusakiewicz S, Kalimutho M. Natural killer cells are essential for the ability of BRAF inhibitors to control BRAFV600E-mutant metastatic melanoma. Cancer Res. 2014; 74: 7298-7308.

19. Gattoni-Celli S, Cole DJ. Melanoma-associated tumor antigens and their clinical relevance to immunotherapy. Semin Oncol. 1996; 23: 754-758.

20. Geertsen R, Hofbauer G, Kamarashev J, Yue FY, Dummer R. Immune escape mechanisms in malignant melanoma. Int J Mol Med. 1999; 3: 49-57.

21. Kalialis LV, Drzewiecki KT, Klyver H. Spontaneous regression of metastases from melanoma: review of the literature. Melanoma Res. 2009; 19: 275-282.

22. Baldea I, Filip A. Photodynamic therapy in melanoma-an update. J Physiol Pharmacol. 2012; 63: 108-119.

23. Isaacs A, Lindenmann J. Virus interference. I. The interferon. Proc R Soc Lond B Biol Sci. 1957; 147: 258-267.

24. Ascierto PA, Chiarion-Sileni V, Muggiano A, MandalÃ M, Pimpinelli N, et al. Interferon alpha for the adjuvant treatment of melanoma: review of international literature and practical recommendations from an expert panel on the use of interferon. Journal of Chemotherapy. 2014; 26:193-201.

25. Kirkwood JM, Richards T, Zarour HM, Sosman J, Ernstoff M, et al. Immunomodulatory effects of high-dose and low-dose interferon Î±2b in patients with high-risk resected melanoma: the E2690 laboratory corollary of intergroup adjuvant trial E1690. Cancer. 2002; 95:1101-1112.

26. Mocellin S, Pasquali S, Rossi CR, Nitti D. Interferon alpha adjuvant therapy in patients with high-risk melanoma: a systematic review and meta-analysis. J Natl Cancer Inst. 2010; 102: 493-501.

27. Wheatley K, Ives N, Eggermont A, Kirkwood J, Cascinelli N, et al. Interferon-{alpha} as adjuvant therapy for melanoma: an individual patient data meta-analysis of randomised trials. In ASCO Annual Meeting Proceedings. 2007; 25: 8526.

28. Dummer R, Hauschild A, Lindenblatt N, Pentheroudakis G, Keilholz U; ESMO Guidelines Committee. Cutaneous melanoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and

follow-up. Ann Oncol. 2015; 26 Suppl 5: v126-132.

29. Garbe C, Radny P, Linse R, Dummer R, Gutzmer R, et al. Adjuvant low-dose interferon (alpha) 2a with or without dacarbazine compared with surgery alone: a prospective randomized phase III DeCOG trial in melanoma patients with regional lymph node metastasis. Ann Oncol. 2008; 19: 1195-1201.

30. Hauschild A, Weichenthal M, Rass K, Linse R, Berking C, et al. Efficacy of low-dose interferon {alpha}2a 18 versus 60 months of treatment in patients with primary melanoma of >1.5 mm tumor thickness: results of a randomized phase III DeCOG trial. J Clin Oncol. 2010; 28: 841-846.

31. Ascierto PA, Kirkwood JM. Adjuvant therapy of melanoma with interferon: lessons of the past decade. J Transl Med. 2008; 6: 62.

32. Moschos SJ, Edington HD, Land SR, Rao UN, Jukic D. Neoadjuvant treatment of regional stage IIIB melanoma with high-dose interferon alfa-2b induces objective tumor regression in association with modulation of tumor infiltrating host cellular immune responses. J Clin Oncol. 2006; 24: 3164-3171.

33. Wang W, Edington HD, Rao UN, Jukic DM, Land SR. Modulation of signal transducers and activators of transcription 1 and 3 signaling in melanoma by high-dose IFNalpha2b. Clin Cancer Res. 2007; 13: 1523-1531.

34. Gogas H, Ioannovich J, Dafni U, Stavropoulou-Giokas C, Frangia K. Prognostic significance of autoimmunity during treatment of melanoma with interferon. N Engl J Med. 2006; 354: 709-718.

35. Kirkwood JM, Ibrahim JG, Sosman JA, Sondak VK, Agarwala SS, et al. High-dose interferon alfa-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/S9512/C509801. J Clin Oncol. 2001; 19: 2370-2380.

36. Yurkovetsky ZR, Kirkwood JM, Edington HD, Marrangoni AM, Velikokhatnaya L. Multiplex analysis of serum cytokines in melanoma patients treated with interferon-alpha2b. Clin Cancer Res. 2007; 13: 2422-2428.

37. Grob JJ, Jouary T, DreÂ´no B, Asselineau J, Gutzmer R, et al. Adjuvant therapy with pegylated interferon alfa-2b (36 months) versus low-dose interferon alfa-2b (18 months) in melanoma patients without macrometastatic nodes: an open-label, randomised, phase 3 European Association for Dermato-Oncology (EADO) study. Eur J Can-

cer. 2013; 49: 166-174.

38. Eggermont AM, Suciu S, Santinami M, Testori A, Kruit WH. Adjuvant therapy with pegylated interferon alfa-2b versus observation alone in resected stage III melanoma: final results of EORTC 1899, a randomised phase III trial. Lancet. 2008; 372: 117-126.

39. Eggermont AM, Suciu S, Testori A, Kruit WH, Marsden J. Ulceration and stage are predictive of interferon efficacy in melanoma: results of the phase III adjuvant trials EORTC 18952 and EORTC 18991. Eur J Cancer. 2012; 48: 218-225.

40. Foletto MC, Haas SE1. Cutaneous melanoma: new advances in treatment. An Bras Dermatol. 2014; 89: 301-310.

41. Weide B, Derhovanessian E, Pflugfelder A, Eigentler TK, Radny P, et al. High response rate after intratumoral treatment with interleukin-2: results from a phase 2 study in 51 patients with metastasized melanoma. Cancer. 2010; 116: 4139-4146.

42. Maverakis E, Cornelius LA, Bowen GM, Phan T, Patel FB. Metastatic melanoma - a review of current and future treatment options. Acta Derm Venereol. 2015; 95: 516-524.

43. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA,, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010; 363: 711-723.

44. Kirkwood JM, Ibrahim JG, Sondak VK, Richards J, Flaherty LE. High- and low-dose interferon alfa-2b in high-risk melanoma: first analysis of intergroup trial E1690/S9111/C9190. J Clin Oncol. 2000; 18: 2444-2458.

45. Kirkwood JM, Strawderman MH, Ernstoff MS, Smith TJ, Borden EC, et al. Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684. Journal of Clinical Oncology. 1996; 14: 7-17.

46. Castano AP, Mroz P, Hamblin MR. Photodynamic therapy and anti-tumour immunity. Nat Rev Cancer. 2006; 6: 535-545.

47. Mroz P, Huang YY, Szokalska A, Zhiyentayev T, Janjua S. Stable synthetic bacteriochlorins overcome the resistance of melanoma to photodynamic therapy. FASEB J. 2010; 24: 3160-3170.

48. Gollnick SO, Evans SS, Baumann H, Owczarczak B, Maier P. Role of cytokines in photodynamic therapy-induced local and systemic inflammation. Br J Cancer. 2003; 88: 1772-1779.

49. Yom SS, Busch TM, Friedberg JS, Wileyto EP, Smith D. Elevated serum cytokine levels in mesothelioma patients who have undergone pleurectomy or extrapleural pneumonectomy and adjuvant intraoperative photodynamic therapy. Photochem Photobiol. 2003; 78: 75-81.

50. Verrico AK, Haylett AK, Moore JV. In vivo expression of the collagen-related heat shock protein HSP47, following hyperthermia or photodynamic therapy. Lasers Med Sci. 2001; 16: 192-198.

51. Hanlon JG, Adams K, Rainbow AJ, Gupta RS, Singh G. Induction of Hsp60 by Photofrin-mediated photodynamic therapy. J Photochem Photobiol B. 2001; 64: 55-61.

52. Gomer CJ, Ferrario A, Rucker N, Wong S, Lee AS. Glucose regulated protein induction and cellular resistance to oxidative stress mediated by porphyrin photosensitization. Cancer Res. 1991; 51: 6574-6579.

53. Korbelik M, Sun J, Cecic I. Photodynamic therapy-induced cell surface expression and release of heat shock proteins: relevance for tumor response. Cancer Res. 2005; 65: 1018-1026.

54. Nowis D, Legat M, Grzela T, Niderla J, Wilczek E. Heme oxygenase-1 protects tumor cells against

photodynamic therapy-mediated cytotoxicity. Oncogene. 2006; 25: 3365-3374.

55. Garg AD, Nowis D, Golab J, Vandenabeele P, Krysko DV. Immunogenic cell death, DAMPs and anticancer therapeutics: an emerging amalgamation. Biochim Biophys Acta. 2010; 1805: 53-71.

56. Michalak M, Groenendyk J, Szabo E, Gold LI, Opas M. Calreticulin, a multi-process calcium-buffering chaperone of the endoplasmic reticulum. Biochem J. 2009; 417: 651-666.

57. Tesniere A, Panaretakis T, Kepp O, Apetoh L, Ghiringhelli F. Molecular characteristics of immunogenic cancer cell death. Cell Death Differ. 2008; 15: 3-12.

58. Kepp O, Tesniere A, Schlemmer F, Michaud M, Senovilla L. Immunogenic cell death modalities and their impact on cancer treatment. Apoptosis. 2009; 14: 364-375.

59. Korbelik M, Sun J, Cecic I. Photodynamic therapy-induced cell surface expression and release of heat shock proteins: relevance for tumor response. Cancer Res. 2005; 65: 1018-1026.

60. Hsieh YJ, Wu CC, Chang CJ, Yu JS. Subcellular localization of Photofrin determines the death phenotype of human epidermoid carcinoma A431 cells triggered by photodynamic therapy: when

plasma membranes are the main targets. J Cell Physiol. 2003; 194: 363-375.

61. Garg AD, Krysko DV, Vandenabeele P, Agostinis P. DAMPs and PDT-mediated photo-oxidative stress: exploring the unknown. Photochem Photobiol Sci. 2011; 10: 670-680.

62. Schmitt E, Gehrmann M, Brunet M, Multhoff G, Garrido C. Intracellular and extracellular functions of heat shock proteins: repercussions in cancer therapy. J Leukoc Biol. 2007; 81: 15-27.

63. Preise D, Oren R, Glinert I, Kalchenko V, Jung S, et al. Systemic antitumor protection by vascular-targeted photodynamic therapy involves cellular and humoral immunity. Cancer Immunol Immunother. 2009; 58:71-84.

64. Campbell DJ, Koch MA. Phenotypical and functional specialization of FOXP3+ regulatory T cells. Nat Rev Immunol. 2011; 11: 119-130.

65. Saji H, Song W, Furumoto K, Kato H, Engleman EG. Systemic antitumor effect of intratumoral injection of dendritic cells in combination with local photodynamic therapy. Clin Cancer Res. 2006; 12: 2568-2574.

66. Jiang H, Granville DJ, North JR, Richter AM, Hunt DW. Selective action of the photosensitizer

QLT0074 on activated human T lymphocytes. Photochem Photobiol. 2002; 76: 224-231.

67. Pasare C, Medzhitov R. Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells. Science. 2003; 299: 1033-1036.

68. Korbelik M, Cooper PD. Potentiation of photodynamic therapy of cancer by complement: the effect of gamma-inulin. Br J Cancer. 2007; 96: 67-72.

69. Mocellin S, Pasquali S, Rossi CR, Nitti D. Interferon alpha adjuvant therapy in patients with high-risk melanoma: a systematic review and meta-analysis. J Natl Cancer Inst. 2010; 102: 493-501.

70. Korbelik M, Sun J. Photodynamic therapy-generated vaccine for cancer therapy. Cancer Immunol Immunother. 2006; 55: 900-909.

71. Gollnick SO, Vaughan L, Henderson BW. Generation of effective antitumor vaccines using photodynamic therapy. Cancer Res. 2002; 62: 1604-1608.

72. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100: 57-70.

73. Jandus C, Bioley G, Speiser DE, Romero P. Selective accumulation of differentiated FOXP3(+) CD4 (+) T cells in metastatic tumor lesions from

melanoma patients compared to peripheral blood. Cancer Immunol Immunother. 2008; 57: 1795-1805.

74. Binder DC, Schreiber H. Dual blockade of PD-1 and CTLA-4 combined with tumor vaccine effectively restores T-cell rejection function in tumors letter. Cancer Res. 2014; 74: 632.

75. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA,, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010; 363: 711-723.

76. Robert C, Thomas L, Bondarenko I, O'Day S, Weber J. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011; 364: 2517-2526.

77. Ribas A, Kefford R, Marshall MA, Punt CJ, Haanen JB. Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. J Clin Oncol. 2013; 31: 616-622.

78. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012; 366: 2443-2454.

79. Robert C, Long GV, Brady B, Dutriaux C, Maio M. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med. 2015; 372: 320-330.

80. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012; 366: 2455-2465.

81. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014; 515: 563-567.

82. Postow MA, Callahan MK, Barker CA, Yamada Y, Yuan J. Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med. 2012; 366: 925-931.

83. Ribas A, Hodi FS, Callahan M, Konto C, Wolchok J. Hepatotoxicity with combination of vemurafenib and ipilimumab. N Engl J Med. 2013; 368: 1365-1366.

84. Hodi FS, Lee S, McDermott DF, Rao UN, Butterfield LH. Ipilimumab plus sargramostim vs ipilimumab alone for treatment of metastatic melanoma: a randomized clinical trial. JAMA. 2014; 312: 1744-1753.

85. Weber JS, Kähler KC, Hauschild A. Management of immune-related adverse events and kinetics of

response with ipilimumab. J Clin Oncol. 2012; 30: 2691-2697.

86. Antoni Ribas, F Stephen Hodi, Richard Kefford, Omid Hamid, Adil Daud, et al. Efficacy and safety of the anti-PD-1 monoclonal antibody MK-3475 in 411 patients (pts) with melanoma (MEL). J Clin Oncol. 2014; 32: Abstract.
87. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res. 2009; 15: 7412-7420.
88. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013; 369: 122-133.