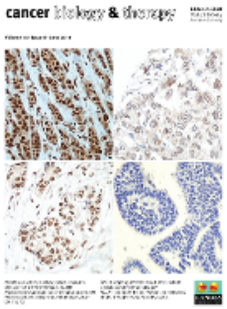
Melanoma immunotherapy.



Cancer Biology & Therapy

ISSN: 1538-4047 (Print) 1555-8576 (Online) Journal homepage: http://www.tandfonline.com/loi/kcbt20

Melanoma immunotherapy

Martina Sanlorenzo, Igor Vujic, Christian Posch, Akshay Dajee, Adam Yen, Sarasa Kim, Michelle Ashworth, Michael D Rosenblum, Alain Algazi, Simona Osella-Abate, Pietro Quaglino, Adil Daud & Susanna Ortiz-Urda

To cite this article: Martina Sanlorenzo, Igor Vujic, Christian Posch, Akshay Dajee, Adam Yen, Sarasa Kim, Michelle Ashworth, Michael D Rosenblum, Alain Algazi, Simona Osella-Abate, Pietro Quaglino, Adil Daud & Susanna Ortiz-Urda (2014) Melanoma immunotherapy, Cancer Biology & Therapy, 15:6, 665-674, DOI: 10.4161/cbt.28555

To link to this article: http://dx.doi.org/10.4161/cbt.28555

Published online: 20 Mar 2014.

Cancer Biology & Therapy 15:6, 665–674; June 2014; © 2014 Landes Bioscience

Melanoma immunotherapy

Martina Sanlorenzo[1,2], Igor Vujic[1,3], Christian Posch[1,3], Akshay Dajee[1], Adam Yen[1], Sarasa Kim[1], Michelle Ashworth[1], Michael D Rosenblum[1], Alain Algazi[1], Simona Osella-Abate[2], Pietro Quaglino[2], Adil Daud[1], and Susanna Ortiz-Urda[1,*]

[1]University of California San Francisco; San Francisco, CA USA; [2]Department of Medical Sciences; Section of Dermatology; University of Turin; Turin, Italy; [3]The Rudolfstiftung Hospital; Vienna, Austria

Keywords: interleukins, interferons, CTLA-4, ipilimumab, PD-1, anti-PD-1, immunoprofiling

Abbreviations: FDA, US Food and Drug Administration; IL-2, interleukin-2; HD IL-2, high-dose interleukin 2; NK, natural killer; CR, complete response; SITC, Society for Immunotherapy for Cancer; NRAS, neuroblastoma rat sarcoma viral oncogene; pDNA, plasmid DNA; IFN-α, interferon-alpha; LAK, lymphocyte-activated killer; BCT, biochemotherapy; GM-CSF, granulocyte-macrophage colony-stimulating factor; ACT, adoptive cell therapy; BRAF, v-Raf murine sarcoma viral oncogene homolog B1; TLR, toll-like receptor; CTLA-4, cytotoxic T-lymphocyte antigen 4; irAEs, immune-related adverse effects; gp100, glycoprotein 100 peptide; OS, overall survival; EORTC, European Organisation for Research and Treatment of Cancer; PD-1, programmed death-1; PDL-1, programmed death ligand 1; LAG-3, lymphocyte activation gene-3; TIM-3, T-cell immunoglobulin mucin-3; ALC, absolute lymphocyte count; ICOS, inducible costimulator molecule; IDO, indoleamine 2,3-dioxygenase

Immunotherapy is a cornerstone in the treatment of melanoma, and is intended to modulate the host immunity against the tumor. Immunotherapy can be used in an adjuvant setting, after complete surgical excision in patients with a high risk of disease relapse and as a treatment in advanced (unresectable or metastatic) stages. Development of novel therapeutic approaches and the optimization of existing therapies hold a great promise in the field of melanoma therapy research. Different clinical trials are ongoing, and immunotherapy is showing the ability to confirm durable clinical benefits in selected groups of melanoma patients. The aim of this review is to summarize different types of immunotherapy agents, as well as to discuss different strategies, complementary regimens, and possible biomarkers of response to the treatment.

Introduction

Melanoma is considered to be one of the best examples of an immunogenic tumor. Different observations have led to this assumption: (1) primary melanomas often exhibit strong lymphocytic infiltration, that could induce partial or complete regression, (2) development of vitiligo is a marker of better prognosis in melanoma patients,[1] and (3) immunotherapies have shown remarkable long-term results.[2,3] The observation that immune response affects tumors biology dates back to the late 1800s, when William B Cooley observed remission of lesions in patients injected with a mixture of dead *Streptococcus pyogenes* and dead *Serratia marcescens* bacteria. Current approaches to cancer immunotherapy include: (1) non-specific stimulation of antitumor immune response by stimulating endogenous effector cells with cytokines, (2) active immunization, (3) adoptive immunotherapy, and (4) targeting of immune checkpoints or immune regulatory molecules (**Fig. 1**). Currently approved melanoma therapeutics by the US Food and Drug Administration (FDA) for the treatment of melanoma, are listed in **Figure 2**. The aim of this review is to summarize different types of immunotherapy agents, as well as to discuss different treatment strategies, complementary regimens, and possible biomarkers of response to the treatment.

*Correspondence to: Susana Ortiz-Urda; Email: ortizsm@derm.ucsf.edu
Submitted: 01/28/2014; Revised: 03/11/2014; Accepted: 03/16/2014;
Published Online: 03/20/2014
http://dx.doi.org/10.4161/cbt.28555

Cytokines

Interleukin-2 (IL-2)

The first type of immunotherapy approved in the treatment of melanoma was high-dose interleukin 2 (HD IL-2), which provided a "proof-of-principle" for the use of immunotherapy in melanoma.[2] Interleukin-2 plays a central role in the activation and stimulation of T lymphocytes and natural killer (NK) cells. In response to IL-2 stimulation these cells acquire cytolytic properties which is believed to enhance their anti-tumoral properties.[4]

IL-2 in advanced melanoma

HD IL-2 is administered ranging from 600000 to 720000 IU/kg/i.v. every 8 h for up to 14 consecutive doses over 5 d, followed by a second treatment cycle after 6 to 9 d.[2] Treatment of patients with advanced melanoma with HD IL-2 has demonstrated a complete response (CR) rate of 6% and partial response rate of 10%. Among patients who reach CR, the response can be long-lasting. However, HD IL-2 is associated with significant acute toxicity (severe hypotension, pulmonary edema, systemic edema with significant weight gain and renal insufficiency, rash and fatigue).[5] For this reason HD IL-2 requires the hospitalization and is usually reserved for patients in a good performance status. Alternative regimens have been investigated, but were unable to reach comparable response rates. The Society for Immunotherapy for Cancer (SITC) recommends HD IL-2 as first-line treatment in patients with stage IV BRAF-wild type

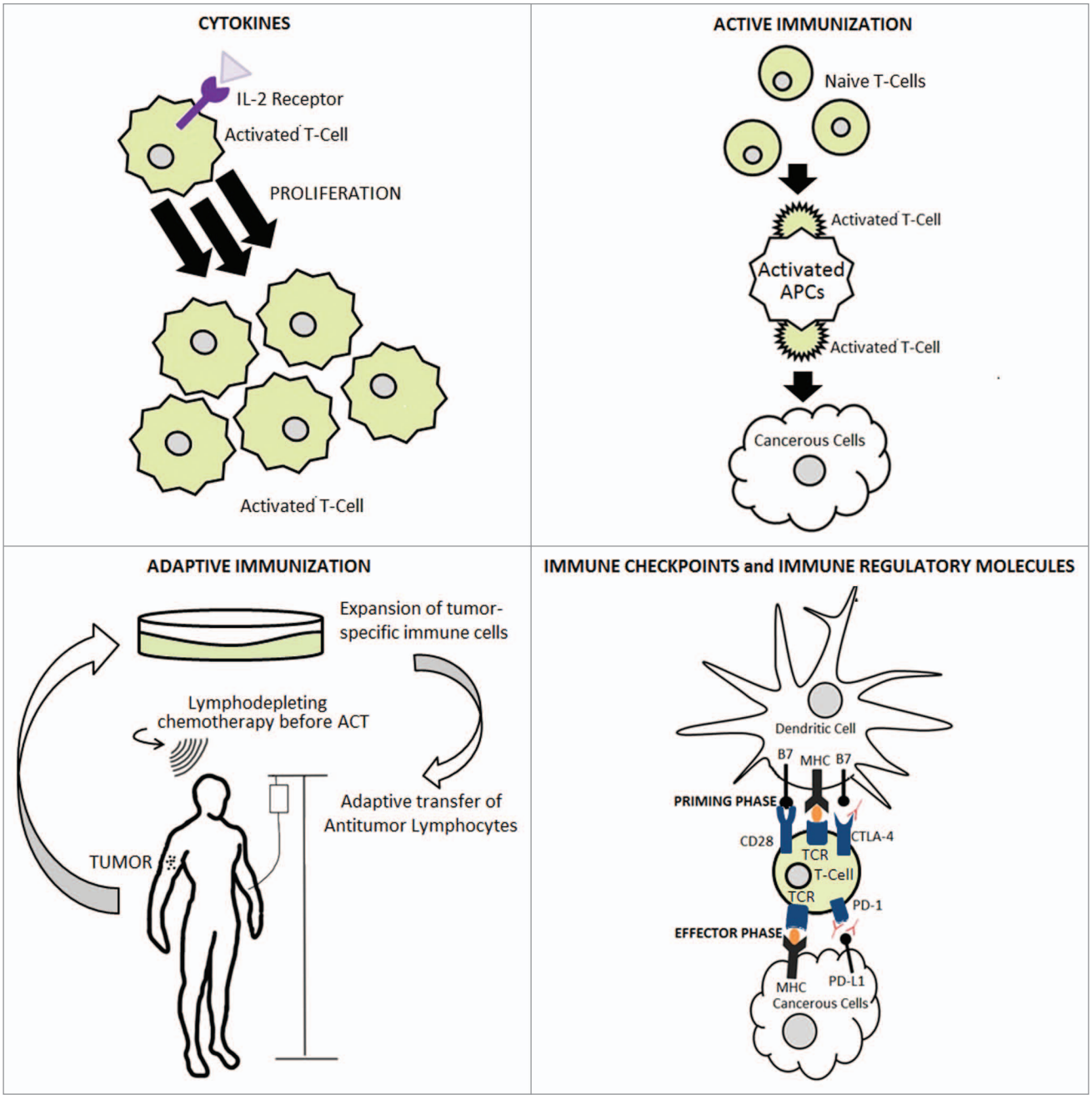


Figure 1. Methods in cancer immunotherapy.

melanoma who have a good performance status and no evidence of central nervous system disease.[6] The genetic background of melanoma might also affect response rates for IL-2 treatment. A recent report[7] suggests that neuroblastoma rat sarcoma viral oncogene (NRAS) mutations might predict a better response to IL-2 treatment.

Intralesional IL-2 for in-transit lesions

Surgical resection is the preferred therapeutic approach for in-transit metastases. However, when surgical excision cannot be pursued, another possible option is the intralesional injection of therapeutics. Injecting IL-2 into a metastatic lesion allows for very high intralesional concentrations without systemic toxicity. Several small series have reported promising clinical responses in treated lesions.[8-11] Boyd et al. injected 10 million IU of IL-2 in each lesion twice a week in a total of 39 patients and reported complete and partial responses in 51% and 31%, respectively.[8] Radny et al. used single doses from 0.6 to 6 million IU, depending on lesion size and injected 2–3 times weekly in a total of 24 patients. They reported complete and partial responses in 63% and 21%, respectively.[10] A systemic effect of this treatment was suggested, with a higher five-year survival in patients with a complete response compared with patients with a partial response (80% vs 50%).[8] To achieve increased local expression of the cytokine over a prolonged period of time, plasmid DNA (pDNA)

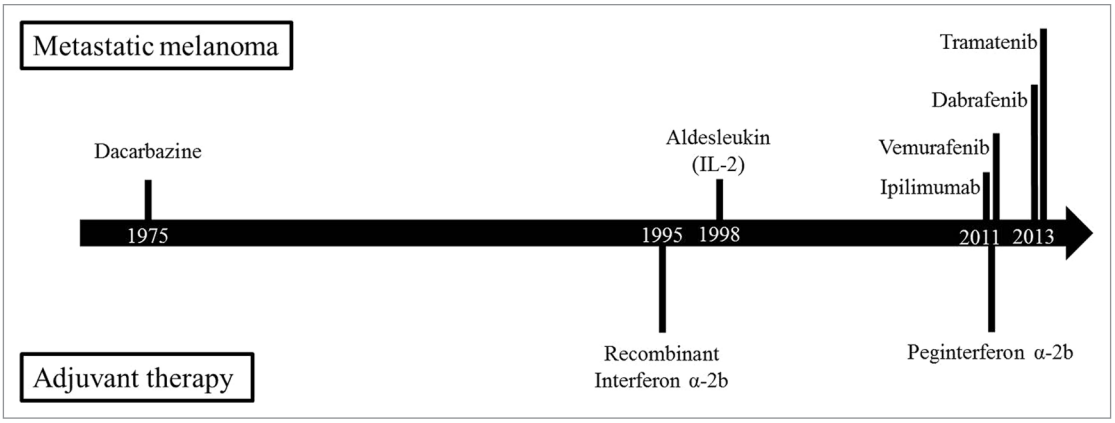


Figure 2. Drugs FDA-approved for the treatment of melanoma with approval date.

expression vectors, which deliver the IL-2 gene into tumor cells, are used.[12] The combination of vector delivery via intralesional injection followed by electroporation to facilitate cellular uptake of pDNA is an area of active research.[13]

Several other immunomodulatory gene therapy trials have been conducted; still the most promising results are achieved with administration of pDNA encoding IL-12 along with electroporation. This treatment has also induced responses in untreated lesions, suggesting an induction of systemic response.[14]

Interferon-α

Interferons are proteins secreted in order to communicate and trigger immune responses to eradicate pathogens and tumor cells. Interferon-α (IFN-α) directly inhibits the proliferation of tumor cells and increases MHC class I expression, enhancing antigen recognition. Moreover, IFN-α represses oncogene and induces tumor suppressor genes expression, in conjunction with antiangiogenic effects.[15] IFN-α is known to regulate the immune system directly or indirectly by the induction of chemokines secretion. In particular, the JAK-STAT pathway plays a critical role in the signaling events induced by IFN, this pathway activates transcription factors STAT, and the activated STAT translocate to the nucleus followed by binding to IFN-stimulated response elements and modulating transcription of IFN-stimulated genes.[16] For example, STAT1 has been proved to be fundamental for the activation of NK cells in response to IFN in murine models.[17] While STAT1 enhances innate and adaptive immunity, STAT3 appears to play opposite role in tumorigenesis, promoting survival, proliferation, motility, and immune tolerance. Studies in STAT-deficient cells have revealed the existence of reciprocal STAT1:STAT3 regulatory mechanisms, and their relative abundance can be implicated in the biological effects in response to IFN activating stimuli.[18]

IFN in adjuvant treatment

Various systemic therapies have been examined for patients with stage II or III melanoma and a high risk of systemic recurrence after surgery excision. The immunotherapeutics approved by the FDA for the adjuvant treatment of melanoma are interferon α-2b and peginterferon α-2b. The rationale for the use of the pegylated form is to reduce its rate of absorption following subcutaneous injection, to reduce renal and cellular clearance, and to decrease the immunogenicity of the protein. All of these effects tend to enhance the half-life of the pegylated protein. On the other hand, pegylation may interfere with the ability of a protein to bind to its receptor, thereby decreasing its biologic effect. Thus, the true biologic effect of the pegylated protein is determined by the balance of these competing properties of the interferon increases.[19]

Stage III melanoma with macroscopic nodal disease (N1b and N2–N3) is generally considered separately from that with microscopically involved lymph nodes (N1a). The SITC suggests one year of interferon α-2b treatment or enrollment in specific clinical trials, for patients with macroscopic lymph node involvement.[6] On the other hand, in the case of N1a disease, a 1-y course of interferon α-2b, but also no further treatment, or shorter courses of interferon α-2b are suggested. Pegylated interferon α-2b was recommended by a minority of panel members in N1a melanoma with ulcerated primary tumors or for patients unwilling or unable to tolerate the standard regimen of interferon α-2b treatment.[6] Patients with stage II melanoma are considered at high-risk of recurrence if the primary lesion is ulcerated, larger than 4 mm in diameter, or has mitotic rate ≥1 per mm^2. Specialists remain divided between recommending interferon α-2b and active surveillance.[6] A recent randomized study showed a better overall survival in patients treated with a 1-y course of interferon α-2b (20 MIU/m^2 intravenously daily 5 d per week for 4 wk, followed by IFN-α-2b 10 MIU/m^2 administered subcutaneously three times per week for 48 wk) compared with a shorter course (20 MIU/m^2 intravenously daily 5 d per week for 4 wk) in patients from stage IIB to stage IIIC.[20] However, most of the patients included had a stage IIIB or IIIC at study entry suggesting once more that 1 y of therapy offers the most benefit to those with macroscopic nodal metastasis and a higher risk of relapse.

Biochemotherapy

In the treatment of metastatic melanoma, the combination of chemotherapy with immunotherapy, biochemotherapy (BCT), has been explored in multiple trials. The most used regimen utilizes cisplatin, vinblastine, and dacarbazine with IL-2 and interferon-α-2b. This combination was designed to enhance immune recognition and effector cell activity triggered by IL-2 during antigen release after tumor cell disruption and apoptosis

induced by cytotoxic chemotherapy. Two systemic reviews showed a higher overall response rate with this regimen compared with chemotherapy, but no significant survival advantage.[21,22] A randomized phase III trial compared BTC to interferon-α-2b as adjuvant therapy in 138 patients with stage 3 melanoma and high risk for recurrence, but didn't detect significant differences in overall survival.[23] Another randomized phase III trial compared BTC to interferon-α-2b as adjuvant therapy in 432 patients with stage III melanoma and found that BTC conferred a statistically significant improvement in relapse free survival compared with interferon; but no discernable difference in overall survival with also an higher occurrence of grade IV toxicity.[24]

Active Immunization

Vaccines

The aim of tumor vaccination is to induce immune recognition with subsequent specific tumor cell eradication. Multiple approaches have been examined, the simplest strategy has been to administer defined tumor-associated antigens as whole proteins or peptide fragments. Most protein- or peptide-based vaccines have lacked significant immunogenicity and were unable to induce a robust immune response in monotherapy. Therefore, the combination of peptide and/or protein vaccines with nonspecific immunologic adjuvants, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) or HD IL-2, has been investigated. These studies have not demonstrated a consistent benefit in overall survival to date.[25] In an effort to induce a more specific tumor response, the administration of customized vaccines, derived from whole cells or cell lysates of patient's tumor have been tested. However, these have also not shown any clinical benefit in randomized controlled trials. Other approaches that have been evaluated include recombinant vectors, which encode either an entire gene or the antigenic epitope,[26] and dendritic cells pulsed with tumor cell RNA, DNA, or cell lysate.[27]

Adoptive Immunotherapy

The adoptive cell therapy (ACT) approach utilizes ex vivo cultured autologous lymphocytes. These lymphocytes can be derived from resected metastasis or from peripheral blood. The optimization of the host environment prior to cell transfer appears to be very important for the success of this procedure: high dose chemotherapy is needed to induce lymphodepletion to eliminate immune regulatory elements (both cellular and humoral) that could affect homing and activity of transferred cells.[28] Recent advances in ACT involve the use of autologous engineered T cells that express T-cell receptors specific for various tumor-associated antigens (such as MART-1-TCG genes), or that secrete specific cytokines.

ACT protocols are currently implemented in patients who failed prior systemic treatment for metastatic melanoma. The most common sites to harvest tumor-infiltrating lymphocytes (TILs) are soft tissue and lung metastases. Recently, several groups published results of phase II clinical trials testing TIL-based ACT.[29-32] The lymphodepleting regimen and the TIL production protocols varied in the different studies. The highest objective response rate (72%) was reported with the use of myeloablative pre-conditioning; whereas the use of $CD8^+$ T cell-enriched TILs in a non-myeloablative setting was associated with the lower response rates (20%). Interestingly, 19 out of 20 patients in one study[33] and all 5 patients in another study,[34] who completely responded to TIL-based ACT, have no evidence of disease after more than 3 and 2 y, respectively. This shows the great potential of ACT regimens in patients with advanced melanoma stages.

However, the toxicity of preparative chemotherapy mandates to investigate factors that predict treatment success. Combining ACT with other therapy approaches, such as ipilimumab or v-Raf murine sarcoma viral oncogene homolog B1 (BRAF) inhibitors, as well as exploring synergistic effects of cytokines, such as IL15, are future directions, and clinical trials are underway (NCT01701674 and NCT01659151).[35]

Immune Checkpoints and Immune Modulator Molecules

The antitumor T-cell activity depends on the dendritic cell maturation stimulus received from the tumor, and on the successful interaction of T-cell co-stimulatory molecules with surface receptors on dendritic cells (**Fig. 3**). New immunotherapies target critical regulatory elements of the interaction between T cells, dendritic cells, and tumor cells. These include anti-CTLA-4 monoclonal antibodies such as ipilimumab, toll-like receptor (TLR) agonists, CD40 agonists, anti-PD-1 or PDL-1 antibodies, and others.

Anti-CTLA-4

The cytotoxic T-lymphocyte antigen 4 (CTLA-4) is a receptor that interacts with CD80 (B7-1) and CD86 (B7-2) and downregulates T-cell response.[36] CD28, although with less affinity than CTLA-4, usually binds to B7-1 and B7-2 leading to T cell activation. CTLA-4 receptors are highly expressed on tumor cell lines and inhibit T-cell responses and "mask" tumors from inducing a host immune response.[37] The excessive CTLA-4 expression on tumor cells represents just one mechanism by which tumors are able to actively suppress and evade T-cell activity. Ipilimumab and tremelimumab (CP-675206) are monoclonal antibodies that block CTLA-4 and allow CD28 to bind to B7-1 receptors, which leads to IL-2 secretion, cytotoxic T-cell activation and proliferation.

Anti-CTLA-4 antibodies in metastatic melanoma

Clinical studies confirm that the CTLA-4 inhibitor ipilimumab can induce durable responses and improve overall survival in patients with advanced melanoma. A phase II study comparing different dose regimens (0.3, 3, or 10 mg/kg IV every 3 wk) established that the best response was obtained with 10 mg/kg. However, at this dose, a significant increase in immune-related adverse effects (irAEs) was observed.[38] In the first phase III trial, ipilimumab +/− glycoprotein 100 peptide (gp100) vaccine was

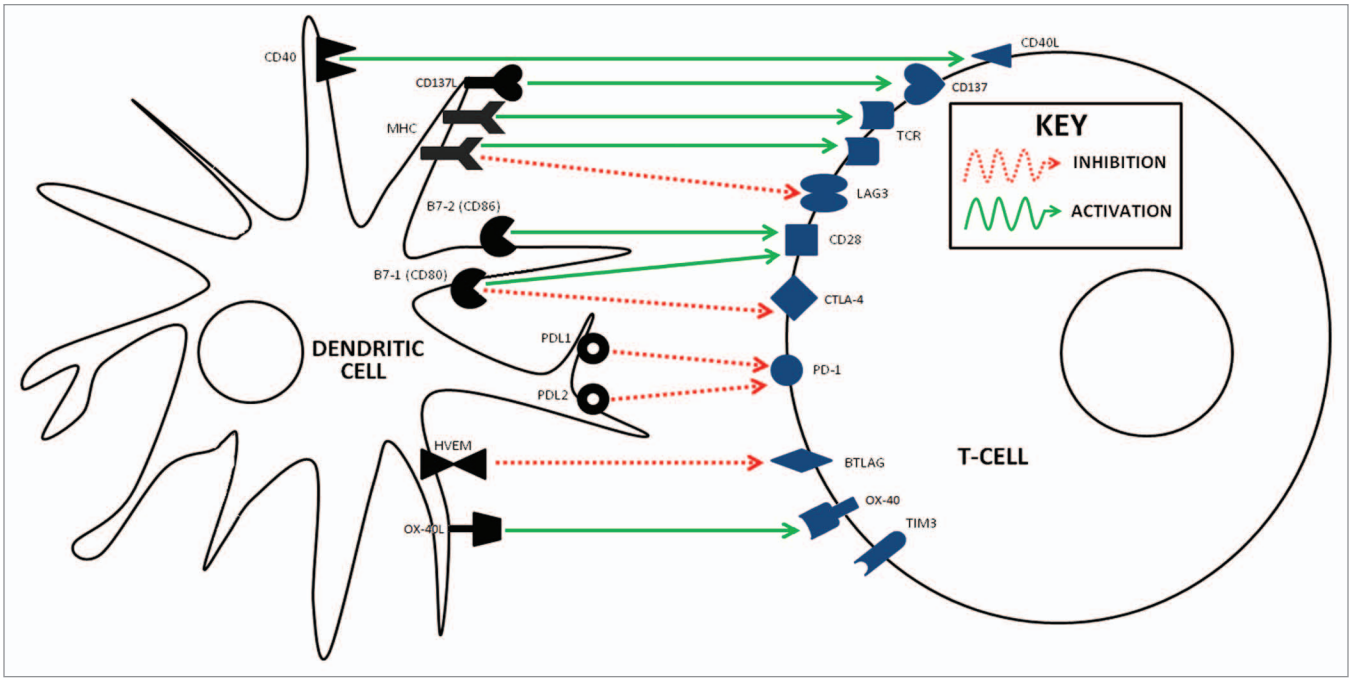


Figure 3. Dendritic cell and T cell interaction.

compared with gp100 vaccine monotherapy in patients with unresectable stage III or stage IV melanoma. Ipilimumab in monotherapy improved overall survival (OS) compared with gp100 vaccine monotherapy (10.1 mo vs. 6.4 mo). However, more irAEs were noticed with ipilimumab treatment. The combination of ipilimumab and gp100 did not improve OS as compared with ipilimumab alone.[39] In another randomized phase III trial ipilimumab (10 mg/kg) combined with dacarbazine (850 mg/mq) demonstrated a modest but statistically significant improvement in OS compared with dacarbazine (850 mg/mq) plus placebo (11.2 mo vs. 9.1 mo). A higher incidence of total adverse events was recorded in the ipilimumab–dacarbazine group compared with dacarbazine–placebo including elevation of alanine aminotransferase levels (in 29.1% of patients vs. 4.4%), elevation of aspartate aminotransferase levels (26.7% vs. 3.2%), diarrhea (32.8% vs. 15.9%), pruritus (29.6% vs. 8.8%), and rash (24.7% vs. 6.8%). Grade 3 or 4 adverse events occurred in 56.3% of patients treated with ipilimumab plus dacarbazine, as compared with 27.5% treated with dacarbazine and placebo ($P < 0.001$).[40]

Subsequently, ipilimumab was approved by the FDA in 2011 for the treatment of unresectable or metastatic melanoma. Similar to other types of immunotherapy, ipilimumab demonstrates durable responses in a small subset of patients. Prieto et al. reported on 177 patients treated with ipilimumab in three different trials. 56 patients received ipilimumab with gp100 peptides, 36 patients received ipilimumab with interleukin-2, and 85 patients received ipilimumab with intrapatient dose-escalation and were randomized to receive gp100 peptides. Out of these 177 patients, 9% experienced a complete response and all but one were durable during follow-up ranging from 54 to 99 mo. Responses were most frequently observed in patients who received a combination of HD-IL2 (720000 IU/kg) and ipilimumab.[41]

First results with tremelimumab showed promising activity in phase I and phase II studies. Although, a subsequent phase III randomized trial comparing tremelimumab to chemotherapy (dacarbazine or temozolamide) in 655 patients with advanced melanoma revealed a not significant prolonged overall survival among patients treated with tremelimumab (11.8 mo with tremelimumab vs. 10.7 mo with chemotherapy, $P = 0.73$).[42] The failure of tremelimumab to improve outcome may be attributed to restrictive inclusion criteria of the study: patients with elevated LDH >2× upper limit of normal, a known negative prognostic indicator, were excluded from the control cohort, making the study populations unbalanced in terms of baseline prognostic factors. This restriction was not present in the ipilimumab phase III trial.[43]

Anti-CTLA-4 antibody in adjuvant therapy

In a phase II trial, 75 patients with resected stage IIIC/IV melanoma were treated with ipilimumab to assess the safety and feasibility of this drug in an adjuvant setting.[44] Improved outcomes in terms of relapse-free survival and overall survival were reported compared with historical data; irAEs were generally reversible and appeared to be associated with improved relapse-free survival.[44] The European Organisation for Research and Treatment of Cancer (EORTC) melanoma group is conducting a phase III trial (EORTC 18071) where resected high-risk patients are randomized to ipilimumab treatment (dose 10 mg/kg every 3 wk for 4 cycles, then every 12 wk for a total of 3 y treatment) or to placebo in order to determine whether adjuvant ipilimumab can prevent disease recurrence. Recruiting has been completed and the results are pending.[35] In addition, a cooperative group trial is currently open to compare adjuvant ipilimumab to high-dose interferon α-2b in treating patients with high-risk stage III or stage IV melanoma (ECOG 1609).[35] Given the minimal impact

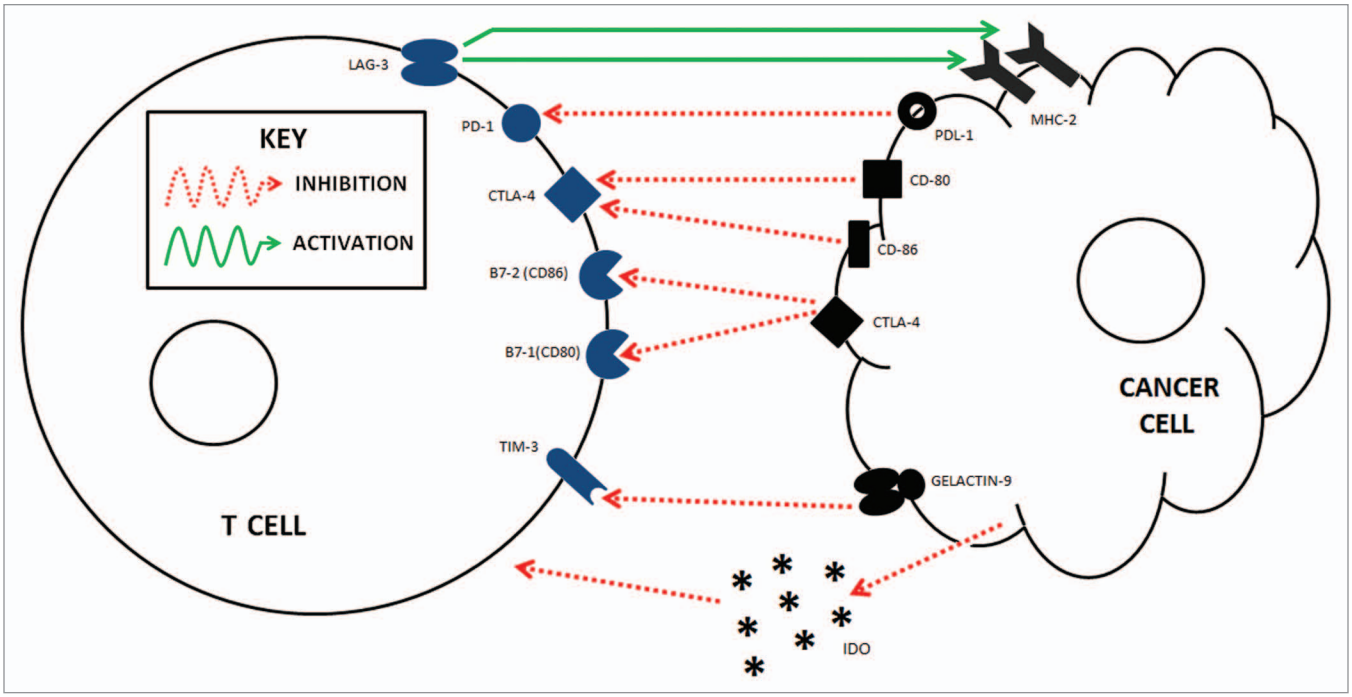


Figure 4. T cell and cancer cell interaction.

of interferon in reducing disease recurrence, this study could provide additional evidence if immunotherapies have the potential to positively influence the immune system to eradicate minimal to microscopic residual disease resulting in prolonged OS.

Anti-PD-1 and PDL-1

Programmed death-1 (PD-1) receptor, also known as CD279, is a protein receptor that is inducibly expressed on $CD4^+$ T cells, $CD8^+$ T cells, natural killer T cells, and B cells. PD-1, interacting with programmed death ligand 1 (PDL-1) and PDL-2 negatively regulates immune responses.[45-47] PD-1 expression usually occurs on T cells during long-term antigen expression and induces T-cell exhaustion. The interaction between tumor cells expressing PD-L1 and T cells expressing PD-1 prevents an effective immune response by inactivating T-cells (**Fig. 4**).

Monoclonal antibodies such as BMS-936558 (nivolumab) and MK-3475 block this immune suppressive interaction of PD-1 and PDL-1. Nivolumab is a human monoclonal antibody targeting PD-1. A phase I study was conducted to test the efficacy and safety of a range of doses of nivolumab in patients with different cancers including 104 melanoma patients. The recommended phase II dose determined for nivolumab was 3 mg/kg IV every 2 wk, and the incidence of irAEs, especially grade 3–4, was lower for patients taking nivolumab compared with the historical data in ipilimumab-treated patients (14% vs 25.2%). Among patients with melanoma treated at the recommended phase II dose of 3 mg/kg, a response was noted in 7 out of 17 patients (41%).[48] Therefore, PD interference seems to display higher response rates and a beneficial side effects profile compared with CTLA-4 inhibition. A clinical trial is currently recruiting patients in order to directly compare efficacy and safety of nivolumab and ipilimumab (NCT01844505).[35]

More recently, a study that combined nivolumab and ipilimumab was completed in patients with advanced melanoma.[49] The concurrent administration of the two drugs every 3 wk, for 4 doses, was compared with a sequenced regimen where patients previously treated with ipilimumab received nivolumab every 2 wk for up to 48 doses. A higher objective-response rate was observed with the concurrent treatment regimen (40% vs 20%). However, grade 3 or 4 side effects occurred in 53% of the patients in the concurrent regimen and in 18% of patients in the sequenced-regimen group. The recommended phase II dosage for this regimen was nivolumab 1 mg/kg with ipilimumab 3 mg/kg.[49]

A second antibody targeting PD-1, MK-3475, also showed promising results with response rates as high as 52%. Among the 57 patients treated with 10 mg/kg IV every 2 wk, 91% experienced irAE but only 23% were grade ≥3.[50] Two more trials have recently been launched to examine the effects of MK-3475 compared with chemotherapy (NCT01704287) and MK-3475 compared with ipilimumab (NCT01866319).[35] Preliminary data from trials with anti-PDL1 drugs (BMS-936559, MPDL3280A) do not seem to as promising as MK-3475 or nivolumab.[51,52]

Other modulators of immune regulatory checkpoints

CD40 agonist

CD40 is a co-stimulatory molecule expressed on dendritic cells, B cells, and monocytes (**Fig. 3**). Its ligand, CD40L, is expressed on $CD4^+$ T cells. The interaction of CD40–CD40L triggers T-cell activation. CD-870873 is a monoclonal antibody targeting CD40. In a phase I study, the administration of CD-870873 was associated with the induction of melanoma antigen-specific T cells, which is believed to be the mechanism of action leading to partial responses which were observed in

27% of patients with metastatic melanoma. The most common adverse event was cytokine release syndrome (grade 1 to 2) which included chills, rigors, and fever.[53] Preclinical data suggest a synergy between chemotherapy and CD40 agonists.[54] In a phase I 32 patients affected by advanced solid tumors were treated with a combination of CP-870 893, paclitaxel and carboplatin. Out of them, 25 patients were affected by metastatic melanoma. Six of the 30 evaluable patients (20%) had a partial response and half of them were melanoma patients.[55] An ongoing trial is recruiting patients with stage IV melanoma in order to assess the most effective dose of CP-870 893 in combination with tremelimumab and the related side effects (NCT01103635).[35]

CD137 agonist

CD137 is an inducible T-lymphocyte surface molecule of the tumor necrosis factor (TNF) receptor superfamily. Its ligand, CD137L, can be expressed by most immune and many non-immune cells as a transmembrane protein (**Fig. 3**). CD137 signaling enhances T-lymphocyte proliferation and T-helper-1 cytokine production, protecting $CD8^+$ T-lymphocytes from apoptosis. A phase I–II study of BMS663513, a human monoclonal antibody agonist of CD137, showed clinical activity with partial remission and stable disease in a subset of patients. However, a high incidence of grade 4 hepatitis was reported and the study had to be discontinued.[56]

Agonistic antibodies targeting OX40

OX40 and its ligand, OX40L (**Fig. 3**), are key TNF members that augment T-cell expansion, cytokine production, and cell survival. OX40 is a co-stimulatory molecule expressed transiently on the surface of T cells. Agonistic antibodies targeting OX40 have been shown to have antitumor activity.[57] A phase I with a proof-of-concept phase II trial was initiated to study in patients with unresectable or metastatic melanoma the combination of OX40 and ipilimumab, but the phase II trial to assess the immune system response to treatment with OX40 antibodies in monotherapy was withdrawn prior to enrollment due to changes in the development plan for the OX40 antibody (NCT01416844).[35]

Anti-LAG-3 antibody

Lymphocyte activation gene-3 (LAG-3) is a transmembrane protein that binds MHC class II molecules, enhances regulatory T-cell activity, and negatively regulates cellular proliferation, activation, and homeostasis of T cells (**Fig. 3**).[58] Further studies revealed a synergy between PD-1 and LAG-3 pathways to induce tolerance to self and tumor antigens.[59] Therefore, the dual blockade of these molecules might be a promising combinatorial strategy for cancer treatment. Moreover, melanoma cells often express MHC class II molecules, and the LAG-3-MHC II interaction protects tumor cells from apoptosis.[59] Targeting LAG-3 could serve as a bidirectional way to prevent tumor immune escape; however, the effects on autoimmunity are yet to be explored. A clinical trial to assess safety of anti-LAG-3 with or without anti-PD-1 in patients with solid tumors is currently recruiting patients (NCT01968109).[35]

Anti-TIM-3 antibody

T-cell immunoglobulin mucin-3 (TIM-3) is another inhibitory receptor expressed on a subset of tumor reactive T cells. Blockage of TIM-3 has been shown to improve the function of anti-tumor T cells in different experimental models (**Fig. 3**). These findings provide the rationale for the clinical development of agents targeting these molecules. Interestingly, TIM-3 is frequently co-expressed with PD-1, and TIM-3/PD-1 expressing cells represented the majority of tumor-infiltrating T cells.[60] Combined targeting of the TIM-3 and PD-1 pathway will be an interesting future treatment approach, but to date clinical trials are not on going yet.

Biomarkers for Immunotherapy

Several immunomodulatory strategies and combinatory approaches involving target inhibitors, immunotherapy, chemotherapy, surgery, and radiation have showed promising results in several clinical trials. However, a significant fraction of patients does not respond to treatment. The development of robust selection criteria and discovery of biomarkers predicting a response to certain treatment strategies is needed to improve response rates, disease-free survival, and OS. Selecting for the right patients will limit the risk of potentially severe irAEs to patients that are most likely to respond, thus greatly improving patient care.

Interleukin-2

Patients with limited subcutaneous and/or cutaneous metastases respond significantly better to IL-2 treatment than patients with other sites of disease.[61] Although high levels of serum VEGF, fibronectin,[62] and C-reactive protein[63] are not established as predictive biomarkers, they have been correlated with a lack of clinical response. A recent report[7] is suggestive of higher clinical responses to HD IL-2 in patients with NRAS mutations (47% responses in NRAS mutant melanoma patients vs 19% in wild type, $P = 0.04$). To date, the majority of predictors for a response to IL-2 treatment have been post-treatment variables, such as magnitude of rebound lymphocytosis, treatment-induced thrombocytopenia, development of autoimmune thyroiditis and vitiligo and decrease in absolute peripheral T regulatory cell count.[64]

Adoptive cell therapy

Retrospective analyses have revealed several factors associated with response in ACT trials. The persistence of transfused cells in circulation after 1 mo,[65-67] the telomere length of infused cells[68] and the elevation of plasmatic levels of cytokines (such as IL-7 and IL-15[69]) were found to correlate with the response to ACT.

Immune-checkpoint regulatory molecules

Several large studies have reported increased efficacy of anti-CTLA-4 in patients who experience irAEs with responses in 26% of patients with irAE, compared with 2% in patients without.[70-72] There was also a "severity response effect" with slightly better response rates for patient grade 3–4 adverse reactions compared with patients with grade 1–2.[70] Several recent studies have shown that an absolute lymphocyte count (ALC) > 1000/μL with an increase in the ALC after 2 ipilimumab treatments correlates with clinical benefit (complete and partial response or stable disease 24 wk after beginning of the treatment) and OS.[72,73] In addition, increased expression of inducible costimulator molecule (ICOS) on T cells and a neutrophil/lymphocytes ratio below the median may represent an early marker of response.[3]

Other approaches trying to predict the response to immunotherapy focus on the tumor microenvironment. A recent study observed improved prognosis among patients with metastatic melanoma when a 12-chemokine gene expression signature (CCL2, CCL3, CCL4, CCL5, CCL8, CCL18, CCL19, CCL21, CXCL9, CXCL10, CXCL11, and CXCL13) was detected in the tumor microenvironment. This chemokine expression profile can be used to predict the likelihood that patients will respond favorably to immunotherapy.[74] A new concept known as immunoprofiling uses the genetically determined or tumor-induced immune response as a predictive factor for the response to immunotherapy.[75] Immunohistochemical analysis of pre- and post-treatment tumor biopsies from patients treated with ipilimumab has shown, that the tumor microenvironment may predict clinical activity. Baseline high expression of FoxP3 and indoleamine 2,3-dioxygenase (IDO), and a high count of tumor-infiltrating lymphocytes at baseline are related to a higher clinical activity of ipilimumab.[76] These biomarkers could help to develop an immunoprofiling panel to select patients that are more likely to respond to anti-CTLA-4 therapy.[75]

Different studies suggest a relationship between PD-L1 expression on tumor cells and objective response to both anti-PD-1 antibodies[48,77] and anti-PD-L1 antibodies.[52] This also suggests that other immune checkpoint regulatory molecules such as anti-OX40, anti-TIM3 among others, may be useful to predict efficacy of therapeutics.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments/Funding

This project was supported by the Melanoma Research Alliance. The study sponsor was not involved in the study design and the collection, analysis, and interpretation of data, nor the writing of the article or the decision to submit it for publication. The authors were independent from the study sponsors.

References

1. Quaglino P, Marenco F, Osella-Abate S, Cappello N, Ortoncelli M, Salomone B, Fierro MT, Savoia P, Bernengo MG. Vitiligo is an independent favourable prognostic factor in stage III and IV metastatic melanoma patients: results from a single-institution hospital-based observational cohort study. Ann Oncol 2010; 21:409-14; PMID:19622589; http://dx.doi.org/10.1093/annonc/mdp325
2. 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(Suppl 1):S11-4; PMID:10685652
3. Di Giacomo AM, Calabrò L, Danielli R, Fonsatti E, Bertocci E, Pesce I, Fazio C, Cutaia O, Giannarelli D, Miracco C, et al. Long-term survival and immunological parameters in metastatic melanoma patients who responded to ipilimumab 10 mg/kg within an expanded access programme. Cancer Immunol Immunother 2013; 62:1021-8; PMID:23591982; http://dx.doi.org/10.1007/s00262-013-1418-6
4. Eklund JW, Kuzel TM. A review of recent findings involving interleukin-2-based cancer therapy. Curr Opin Oncol 2004; 16:542-6; PMID:15627015; http://dx.doi.org/10.1097/01.cco.0000142070.45097.68
5. Mekhail T, Wood L, Bukowski R. Interleukin-2 in cancer therapy: uses and optimum management of adverse effects. BioDrugs 2000; 14:299-318; PMID:18034575; http://dx.doi.org/10.2165/00063030-200014050-00003
6. Kaufman HL, Kirkwood JM, Hodi FS, Agarwala S, Amatruda T, Bines SD, Clark JI, Curti B, Ernstoff MS, Gajewski T, et al. The Society for Immunotherapy of Cancer consensus statement on tumour immunotherapy for the treatment of cutaneous melanoma. Nat Rev Clin Oncol 2013
7. Joseph RW, Sullivan RJ, Harrell R, Stemke-Hale K, Panka D, Manoukian G, Percy A, Bassett RL, Ng CS, Radvanyi L, et al. Correlation of NRAS mutations with clinical response to high-dose IL-2 in patients with advanced melanoma. J Immunother 2012; 35:66-72; PMID:22130161; http://dx.doi.org/10.1097/CJI.0b013e3182372636
8. Boyd KU, Wehrli BM, Temple CLF. Intra-lesional interleukin-2 for the treatment of in-transit melanoma. J Surg Oncol 2011; 104:711-7; PMID:21744347; http://dx.doi.org/10.1002/jso.21968
9. Grotz TE, Mansfield AS, Kottschade LA, Erickson LA, Otley CC, Markovic SN, Jakub JW. In-transit melanoma: an individualized approach. Oncology (Williston Park) 2011; 25:1340-8; PMID:22329185
10. Radny P, Caroli UM, Bauer J, Paul T, Schlegel C, Eigentler TK, Weide B, Schwarz M, Garbe C. Phase II trial of intralesional therapy with interleukin-2 in soft-tissue melanoma metastases. Br J Cancer 2003; 89:1620-6; PMID:14583759; http://dx.doi.org/10.1038/sj.bjc.6601320
11. Ridolfi L, Ridolfi R, Ascari-Raccagni A, Fabbri M, Casadei S, Gatti A, Trevisan G, Righini MG. Intralesional granulocyte-monocyte colony-stimulating factor followed by subcutaneous interleukin-2 in metastatic melanoma: a pilot study in elderly patients. J Eur Acad Dermatol Venereol 2001; 15:218-23; PMID:11683284; http://dx.doi.org/10.1046/j.1468-3083.2001.00254.x
12. Parker SE, Khatibi S, Margalith M, Anderson D, Yankauckas M, Gromkowski SH, Latimer T, Lew D, Marquet M, Manthorpe M, et al. Plasmid DNA gene therapy: studies with the human interleukin-2 gene in tumor cells in vitro and in the murine B16 melanoma model in vivo. Cancer Gene Ther 1996; 3:175-85; PMID:8725882
13. Heller R, Shirley S, Guo S, Donate A, Heller L. Electroporation based gene therapy–from the bench to the bedside. Conf Proc Annu Int Conf IEEE Eng Med Biol Soc IEEE Eng Med Biol Soc Conf 2011; 2011:736–8.
14. Daud AI, DeConti RC, Andrews S, Urbas P, Riker AI, Sondak VK, Munster PN, Sullivan DM, Ugen KE, Messina JL, et al. Phase I trial of interleukin-12 plasmid electroporation in patients with metastatic melanoma. J Clin Oncol 2008; 26:5896-903; PMID:19029422
15. Hervas-Stubbs S, Perez-Gracia JL, Rouzaut A, Sanmamed MF, Le Bon A, Melero I. Direct effects of type I interferons on cells of the immune system. Clin Cancer Res 2011; 17:2619-27; PMID:21372217; http://dx.doi.org/10.1158/1078-0432.CCR-10-1114
16. Sarasin-Filipowicz M, Oakeley EJ, Duong FHT, Christen V, Terracciano L, Filipowicz W, Heim MH. Interferon signaling and treatment outcome in chronic hepatitis C. Proc Natl Acad Sci U S A 2008; 105:7034-9; PMID:18467494; http://dx.doi.org/10.1073/pnas.0707882105
17. Lee C-K, Rao DT, Gertner R, Gimeno R, Frey AB, Levy DE. Distinct requirements for IFNs and STAT1 in NK cell function. J Immunol 2000; 165:3571-7; PMID:11034357
18. Avalle L, Pensa S, Regis G, Novelli F, Poli V. STAT1 and STAT3 in tumorigenesis: A matter of balance. JAKSTAT 2012; 1:65-72; PMID:24058752; http://dx.doi.org/10.4161/jkst.20045
19. Baker DE. Pegylated interferons. Rev Gastroenterol Disord 2001; 1:87-99; PMID:12120178
20. Payne MJ, Argyropoulou K, Lorigan P, McAleer JJ, Farrugia D, Davidson N, Kelly C, Chao D, Marshall E, Han C, et al. Phase II pilot study of intravenous high-dose interferon with or without maintenance treatment in melanoma at high risk of recurrence. J Clin Oncol 2014; 32:185-90; PMID:24344211; http://dx.doi.org/10.1200/JCO.2013.49.8717
21. Sasse AD, Sasse EC, Clark LGO, Ulloa L, Clark OAC. Chemoimmunotherapy versus chemotherapy for metastatic malignant melanoma. Cochrane Database Syst Rev 2007; CD005413; PMID:17253556
22. Ives NJ, Stowe RL, Lorigan P, Wheatley K. Chemotherapy compared with biochemotherapy for the treatment of metastatic melanoma: a meta-analysis of 18 trials involving 2,621 patients. J Clin Oncol 2007; 25:5426-34; PMID:18048825; http://dx.doi.org/10.1200/JCO.2007.12.0253
23. Kim KB, Legha SS, Gonzalez R, Anderson CM, Johnson MM, Liu P, Papadopoulos NE, Eton O, Plager C, Buzaid AC, et al. A randomized phase III trial of biochemotherapy versus interferon-alpha-2b for adjuvant therapy in patients at high risk for melanoma recurrence. Melanoma Res 2009; 19:42-9; PMID:19430405; http://dx.doi.org/10.1097/CMR.0b013e328314b84a
24. Flaherty LE, Moon J, Atkins MB, Tuthill R, Thompson JA, Vetto JT, Haluska FG, Pappo AS, Sosman JA, Redman BG, et al. Phase III trial of high-dose interferon alpha-2b versus cisplatin, vinblastine, DTIC plus IL-2 and interferon in patients with high-risk melanoma (SWOG S0008): An intergroup study of CALGB, COG, ECOG, and SWOG. J Clin Oncol [Internet] 2012 [cited 2014 Jan 21]; 30. Available from: http://meetinglibrary.asco.org/content/92968-114
25. Alexandrescu DT, Ichim TE, Riordan NH, Marincola FM, Di Nardo A, Kabigting FD, Dasanu CA. Immunotherapy for melanoma: current status and perspectives. J Immunother 2010; 33:570-90; PMID:20551839; http://dx.doi.org/10.1097/CJI.0b013e3181e032e8

26. Rosenberg SA, Zhai Y, Yang JC, Schwartzentruber DJ, Hwu P, Marincola FM, Topalian SL, Restifo NP, Seipp CA, Einhorn JH, et al. Immunizing patients with metastatic melanoma using recombinant adenoviruses encoding MART-1 or gp100 melanoma antigens. J Natl Cancer Inst 1998; 90:1894-900; PMID:9862627; http://dx.doi.org/10.1093/jnci/90.24.1894
27. Thurner B, Haendle I, Röder C, Dieckmann D, Keikavoussi P, Jonuleit H, Bender A, Maczek C, Schreiner D, von den Driesch P, et al. Vaccination with mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. J Exp Med 1999; 190:1669-78; PMID:10587357; http://dx.doi.org/10.1084/jem.190.11.1669
28. Rosenberg SA. Cell transfer immunotherapy for metastatic solid cancer--what clinicians need to know. Nat Rev Clin Oncol 2011; 8:577-85; PMID:21808266; http://dx.doi.org/10.1038/nrclinonc.2011.116
29. Besser MJ, Shapira-Frommer R, Treves AJ, Zippel D, Itzhaki O, Hershkovitz L, Levy D, Kubi A, Hovav E, Chermoshniuk N, et al. Clinical responses in a phase II study using adoptive transfer of short-term cultured tumor infiltration lymphocytes in metastatic melanoma patients. Clin Cancer Res 2010; 16:2646-55; PMID:20406835; http://dx.doi.org/10.1158/1078-0432.CCR-10-0041
30. Dudley ME, Yang JC, Sherry R, Hughes MS, Royal R, Kammula U, Robbins PF, Huang J, Citrin DE, Leitman SF, et al. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. J Clin Oncol 2008; 26:5233-9; PMID:18809613; http://dx.doi.org/10.1200/JCO.2008.16.5449
31. Dudley ME, Gross CA, Somerville RPT, Hong Y, Schaub NP, Rosati SF, White DE, Nathan D, Restifo NP, Steinberg SM, et al. Randomized selection design trial evaluating CD8+-enriched versus unselected tumor-infiltrating lymphocytes for adoptive cell therapy for patients with melanoma. J Clin Oncol 2013; 31:2152-9; PMID:23650429; http://dx.doi.org/10.1200/JCO.2012.46.6441
32. Radvanyi LG, Bernatchez C, Zhang M, Fox PS, Miller P, Chacon J, Wu R, Lizee G, Mahoney S, Alvarado G, et al. Specific lymphocyte subsets predict response to adoptive cell therapy using expanded autologous tumor-infiltrating lymphocytes in metastatic melanoma patients. Clin Cancer Res 2012; 18:6758-70; PMID:23032743; http://dx.doi.org/10.1158/1078-0432.CCR-12-1177
33. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, Citrin DE, Restifo NP, Robbins PF, Wunderlich JR, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res 2011; 17:4550-7; PMID:21498393; http://dx.doi.org/10.1158/1078-0432.CCR-11-0116
34. Besser MJ, Shapira-Frommer R, Itzhaki O, Treves AJ, Zippel DB, Levy D, Kubi A, Shoshani N, Zikich D, Ohayon Y, et al. Adoptive transfer of tumor-infiltrating lymphocytes in patients with metastatic melanoma: intent-to-treat analysis and efficacy after failure to prior immunotherapies. Clin Cancer Res 2013; 19:4792-800; PMID:23690483; http://dx.doi.org/10.1158/1078-0432.CCR-13-0380
35. Home - ClinicalTrials.gov [Internet]. [cited 2013 Sep 18]; Available from: http://clinicaltrials.gov/
36. Pentcheva-Hoang T, Corse E, Allison JP. Negative regulators of T-cell activation: potential targets for therapeutic intervention in cancer, autoimmune disease, and persistent infections. Immunol Rev 2009; 229:67-87; PMID:19426215; http://dx.doi.org/10.1111/j.1600-065X.2009.00763.x
37. Shah KV, Chien AJ, Yee C, Moon RT. CTLA-4 is a direct target of Wnt/beta-catenin signaling and is expressed in human melanoma tumors. J Invest Dermatol 2008; 128:2870-9; PMID:18563180; http://dx.doi.org/10.1038/jid.2008.170
38. Wolchok JD, Neyns B, Linette G, Negrier S, Lutzky J, Thomas L, Waterfield W, Schadendorf D, Smylie M, Guthrie T Jr., et al. Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study. Lancet Oncol 2010; 11:155-64; PMID:20004617; http://dx.doi.org/10.1016/S1470-2045(09)70334-1
39. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010; 363:711-23; PMID:20525992; http://dx.doi.org/10.1056/NEJMoa1003466
40. Robert C, Thomas L, Bondarenko I, O'Day S, M D JW, Garbe C, Lebbe C, Baurain JF, Testori A, Grob JJ, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 2011; 364:2517-26; PMID:21639810; http://dx.doi.org/10.1056/NEJMoa1104621
41. Prieto PA, Yang JC, Sherry RM, Hughes MS, Kammula US, White DE, Levy CL, Rosenberg SA, Phan GQ. CTLA-4 blockade with ipilimumab: long-term follow-up of 177 patients with metastatic melanoma. Clin Cancer Res 2012; 18:2039-47; PMID:22271879; http://dx.doi.org/10.1158/1078-0432.CCR-11-1823
42. Ribas A, Kefford R, Marshall MA, Punt CJA, Haanen JB, Marmol M, Garbe C, Gogas H, Schachter J, Linette G, et al. Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. J Clin Oncol 2013; 31:616-22; PMID:23295794; http://dx.doi.org/10.1200/JCO.2012.44.6112
43. Simeone E, Ascierto PA. Immunomodulating antibodies in the treatment of metastatic melanoma: the experience with anti-CTLA-4, anti-CD137, and anti-PD1. J Immunotoxicol 2012; 9:241-7; PMID:22524673; http://dx.doi.org/10.3109/1547691X.2012.678021
44. Sarnaik AA, Yu B, Yu D, Morelli D, Hall M, Bogle D, Yan L, Targan S, Solomon J, Nichol G, et al. Extended dose ipilimumab with a peptide vaccine: immune correlates associated with clinical benefit in patients with resected high-risk stage IIIc/IV melanoma. Clin Cancer Res 2011; 17:896-906; PMID:21106722; http://dx.doi.org/10.1158/1078-0432.CCR-10-2463
45. Nishimura H, Honjo T. PD-1: an inhibitory immunoreceptor involved in peripheral tolerance. Trends Immunol 2001; 22:265-8; PMID:11323285; http://dx.doi.org/10.1016/S1471-4906(01)01888-9
46. Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. Int Immunol 2007; 19:813-24; PMID:17606980; http://dx.doi.org/10.1093/intimm/dxm057
47. Wherry EJ. T cell exhaustion. Nat Immunol 2011; 12:492-9; PMID:21739672; http://dx.doi.org/10.1038/ni.2035
48. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012; 366:2443-54; PMID:22658127; http://dx.doi.org/10.1056/NEJMoa1200690
49. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, Segal NH, Ariyan CE, Gordon R-A, Reed K, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 2013; 369:122-33; PMID:23724867; http://dx.doi.org/10.1056/NEJMoa1302369
50. Hamid O, Robert C, Daud A, Hodi FS, Hwu W-J, Kefford R, Wolchok JD, Hersey P, Joseph RW, Weber JS, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med 2013; 369:134-44; PMID:23724846; http://dx.doi.org/10.1056/NEJMoa1305133
51. Brahmer JR, Tykodi SS, Chow LQM, Hwu W-J, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012; 366:2455-65; PMID:22658128; http://dx.doi.org/10.1056/NEJMoa1200694
52. Hamid O, Sosman JA, Lawrence DP, Sullivan RJ, Ibrahim N, Kluger HM, Boasberg PD, Flaherty K, Hwu P, Ballinger M, et al. Clinical activity, safety, and biomarkers of MPDL3280A, an engineered PD-L1 antibody in patients with locally advanced or metastatic melanoma (mM). J Clin Oncol [Internet] 2013 [cited 2013 Sep 18]; 31. Available from: http://meetinglibrary.asco.org/content/115916-132
53. Vonderheide RH, Flaherty KT, Khalil M, Stumacher MS, Bajor DL, Hutnick NA, Sullivan P, Mahany JJ, Gallagher M, Kramer A, et al. Clinical activity and immune modulation in cancer patients treated with CP-870,893, a novel CD40 agonist monoclonal antibody. J Clin Oncol 2007; 25:876-83; PMID:17327609; http://dx.doi.org/10.1200/JCO.2006.08.3311
54. Nowak AK, Robinson BWS, Lake RA. Synergy between chemotherapy and immunotherapy in the treatment of established murine solid tumors. Cancer Res 2003; 63:4490-6; PMID:12907622
55. Vonderheide RH, Burg JM, Mick R, Trosko JA, Li D, Shaik MN, Tolcher AW, Hamid O. Phase I study of the CD40 agonist antibody CP-870,893 combined with carboplatin and paclitaxel in patients with advanced solid tumors. Oncoimmunology 2013; 2:e23033; PMID:23483678; http://dx.doi.org/10.4161/onci.23033
56. Ascierto PA, Simeone E, Sznol M, Fu Y-X, Melero I. Clinical experiences with anti-CD137 and anti-PD1 therapeutic antibodies. Semin Oncol 2010; 37:508-16; PMID:21074066; http://dx.doi.org/10.1053/j.seminoncol.2010.09.008
57. Weinberg AD, Morris NP, Kovacsovics-Bankowski M, Urba WJ, Curti BD. Science gone translational: the OX40 agonist story. Immunol Rev 2011; 244:218-31; PMID:22017441; http://dx.doi.org/10.1111/j.1600-065X.2011.01069.x
58. Grosso JF, Goldberg MV, Getnet D, Bruno TC, Yen H-R, Pyle KJ, Hipkiss E, Vignali DAA, Pardoll DM, Drake CG. Functionally distinct LAG-3 and PD-1 subsets on activated and chronically stimulated CD8 T cells. J Immunol 2009; 182:6659-69; PMID:19454660; http://dx.doi.org/10.4049/jimmunol.0804211
59. Hemon P, Jean-Louis F, Ramgolam K, Brignone C, Viguier M, Bachelez H, Triebel F, Charron D, Aoudjit F, Al-Daccak R, et al. MHC class II engagement by its ligand LAG-3 (CD223) contributes to melanoma resistance to apoptosis. J Immunol 2011; 186:5173-83; PMID:21441454; http://dx.doi.org/10.4049/jimmunol.1002050
60. Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, Anderson AC. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. J Exp Med 2010; 207:2187-94; PMID:20819927; http://dx.doi.org/10.1084/jem.20100643
61. Phan GQ, Attia P, Steinberg SM, White DE, Rosenberg SA. Factors associated with response to high-dose interleukin-2 in patients with metastatic melanoma. J Clin Oncol 2001; 19:3477-82; PMID:11481353

62. Sabatino M, Kim-Schulze S, Panelli MC, Stroncek D, Wang E, Taback B, Kim DW, Deraffele G, Pos Z, Marincola FM, et al. Serum vascular endothelial growth factor and fibronectin predict clinical response to high-dose interleukin-2 therapy. J Clin Oncol 2009; 27:2645-52; PMID:19364969; http://dx.doi.org/10.1200/JCO.2008.19.1106
63. Tartour E, Blay JY, Dorval T, Escudier B, Mosseri V, Douillard JY, Deneux L, Gorin I, Negrier S, Mathiot C, et al. Predictors of clinical response to interleukin-2--based immunotherapy in melanoma patients: a French multiinstitutional study. J Clin Oncol 1996; 14:1697-703; PMID:8622090
64. Kirkwood JM, Tarhini AA. Biomarkers of therapeutic response in melanoma and renal cell carcinoma: potential inroads to improved immunotherapy. J Clin Oncol 2009; 27:2583-5; PMID:19364958; http://dx.doi.org/10.1200/JCO.2008.21.1540
65. Robbins PF, Dudley ME, Wunderlich J, El-Gamil M, Li YF, Zhou J, Huang J, Powell DJ Jr., Rosenberg SA. Cutting edge: persistence of transferred lymphocyte clonotypes correlates with cancer regression in patients receiving cell transfer therapy. J Immunol 2004; 173:7125-30; PMID:15585832
66. Huang J, Khong HT, Dudley ME, El-Gamil M, Li YF, Rosenberg SA, Robbins PF. Survival, persistence, and progressive differentiation of adoptively transferred tumor-reactive T cells associated with tumor regression. J Immunother 2005; 28:258-67; PMID:15838383; http://dx.doi.org/10.1097/01.cji.0000158855.92792.7a
67. Powell DJ Jr., Dudley ME, Robbins PF, Rosenberg SA. Transition of late-stage effector T cells to CD27+ CD28+ tumor-reactive effector memory T cells in humans after adoptive cell transfer therapy. Blood 2005; 105:241-50; PMID:15345595; http://dx.doi.org/10.1182/blood-2004-06-2482
68. Zhou J, Shen X, Huang J, Hodes RJ, Rosenberg SA, Robbins PF. Telomere length of transferred lymphocytes correlates with in vivo persistence and tumor regression in melanoma patients receiving cell transfer therapy. J Immunol 2005; 175:7046-52; PMID:16272366
69. Gattinoni L, Finkelstein SE, Klebanoff CA, Antony PA, Palmer DC, Spiess PJ, Hwang LN, Yu Z, Wrzesinski C, Heimann DM, et al. Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific CD8+ T cells. J Exp Med 2005; 202:907-12; PMID:16203864; http://dx.doi.org/10.1084/jem.20050732
70. Downey SG, Klapper JA, Smith FO, Yang JC, Sherry RM, Royal RE, Kammula US, Hughes MS, Allen TE, Levy CL, et al. Prognostic factors related to clinical response in patients with metastatic melanoma treated by CTL-associated antigen-4 blockade. Clin Cancer Res 2007; 13:6681-8; PMID:17982122; http://dx.doi.org/10.1158/1078-0432.CCR-07-0187
71. Attia P, Phan GQ, Maker AV, Robinson MR, Quezado MM, Yang JC, Sherry RM, Topalian SL, Kammula US, Royal RE, et al. Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. J Clin Oncol 2005; 23:6043-53; PMID:16087944; http://dx.doi.org/10.1200/JCO.2005.06.205
72. Ku GY, Yuan J, Page DB, Schroeder SEA, Panageas KS, Carvajal RD, Chapman PB, Schwartz GK, Allison JP, Wolchok JD. Single-institution experience with ipilimumab in advanced melanoma patients in the compassionate use setting: lymphocyte count after 2 doses correlates with survival. Cancer 2010; 116:1767-75; PMID:20143434; http://dx.doi.org/10.1002/cncr.24951
73. Callahan MK, Wolchok JD, Allison JP. Anti-CTLA-4 antibody therapy: immune monitoring during clinical development of a novel immunotherapy. Semin Oncol 2010; 37:473-84; PMID:21074063; http://dx.doi.org/10.1053/j.seminoncol.2010.09.001
74. Messina JL, Fenstermacher DA, Eschrich S, Qu X, Berglund AE, Lloyd MC, Schell MJ, Sondak VK, Weber JS, Mulé JJ. 12-Chemokine gene signature identifies lymph node-like structures in melanoma: potential for patient selection for immunotherapy? Sci Rep 2012; 2:765; PMID:23097687; http://dx.doi.org/10.1038/srep00765
75. Ascierto PA, Capone M, Urba WJ, Bifulco CB, Botti G, Lugli A, Marincola FM, Ciliberto G, Galon J, Fox BA. The additional facet of immunoscore: immunoprofiling as a possible predictive tool for cancer treatment. J Transl Med 2013; 11:54; PMID:23452415; http://dx.doi.org/10.1186/1479-5876-11-54
76. Hamid O, Schmidt H, Nissan A, Ridolfi L, Aamdal S, Hansson J, Guida M, Hyams DM, Gómez H, Bastholt L, et al. A prospective phase II trial exploring the association between tumor microenvironment biomarkers and clinical activity of ipilimumab in advanced melanoma. J Transl Med 2011; 9:204; PMID:22123319; http://dx.doi.org/10.1186/1479-5876-9-204
77. Weber JS, Kudchadkar RR, Gibney GT, Conti RCD, Yu B, Wang W, Sarnaik A, Martinez AJ, Kroeger J, Eysmans C, et al. Phase I/II trial of PD-1 antibody nivolumab with peptide vaccine in patients naive to or that failed ipilimumab. J Clin Oncol [Internet] 2013 [cited 2013 Sep 18]; 31. Available from: http://meetinglibrary.asco.org/content/112287-132