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Immunotherapy for Melanoma: Current Status and Perspectives

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Summary

Immunotherapy is an important modality in the therapy of patients with malignant melanoma. As our knowledge about this disease continues to expand, so does the immunotherapeutic armamentarium. Nevertheless, successful preclinical models do not always translate into clinically meaningful results. The authors give a comprehensive analysis of most recent advances in the immune anti-melanoma therapy, including interleukins, interferons, other cytokines, adoptive immunotherapy, biochemotherapy, as well as the use of different vaccines. We also present the fundamental concepts behind various immune enhancement strategies, passive immunotherapy, as well as the use of immune adjuvants. This review brings into discussion the results of newer and older clinical trials, as well as potential limitations and drawbacks seen with the utilization of various immune therapies in malignant melanoma. Development of novel therapeutic approaches, along with optimization of existing therapies, continues to hold a great promise in the field of melanoma therapy research. Use of anti-CTLA4 and anti-PD1 antibodies, realization of the importance of co-stimulatory signals, which translated into the use of agonist CD40 monoclonal antibodies, as well as activation of innate immunity through enhanced expression of co-stimulatory molecules on the surface of dendritic cells by TLR agonists are only a few items on the list of recent advances in the treatment of melanoma. The need to engineer better immune interactions and to boost positive feedback loops appear crucial for the future of melanoma therapy, which ultimately resides in our understanding of the complexity of immune responses in this disease.

Keywords

malignant melanoma; immunotherapy; vaccines; cytokines; immunomodulation; dendritic cells

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FUNDAMENTAL DISCOVERIES AND PERSPECTIVES IN ANTI-TUMOR IMMUNOTHERAPY

Most of the discoveries in human cancer immunology originate from studies of melanoma, a cancer shown to be among the most immunogenic of all tumors. In the past thirty years, much has been learned about the immunobiology of melanoma. As this knowledge continues to expand, so does the potential therapeutic role of immunotherapy in augmenting the antitumor immune responses against melanoma. A schematic representation of the antitumor immune responses generated in melanoma is presented in Figure 1.

Melanoma was the first tumor model to reveal CD4 and CD8 cellular specificity to the tumor differentiation antigens gp100 and tyrosinase.[1,2] The subsequent efforts to identify specific genes encoding tumor antigens and their corresponding epitopes yielded major progress in further understanding of the antitumoral immune responses. It became clear that genetic changes in cancer cells can lead to the build-up of new specific antigens, which are MHC-restricted and recognized by the $CD4^+$ lymphocytes. MAGE-1 represented the first tumor antigen specifically recognized by the cytotoxic $CD8^+$ lymphocytes.[3] Initial studies on MAGE-1 supported the idea that the human immune system could respond to the tumor antigens, thus sparking a great deal of interest in identifying potential therapeutic targets and biomarkers predicting response to immunotherapy. These advances have contributed to the development of vaccines, biological agents such as inter-leukins and interferons, cellular therapies, and antibodies currently in use to treat melanoma. These therapies continue to be tested, either alone or in combination, in order to improve the largely disappointing tumor response rates (RRs) ranging only 5% to 10%.

The fact that successful preclinical studies do not always translate into clinically meaningful objective RRs in patients with melanoma has been a common theme. Although such therapies as vaccines are able to significantly induce tumor antigen-specific T-cells, it has only translated into marginal clinical responses, and often at the cost of severe or life-threatening autoimmune toxicities. The fact that specific cytotoxic T-cells are not capable of efficient tumor lysis led to the concept of tumor tolerance.[4] It is now clear that various immunosuppressive elements in the tumor microenvironment limit the anti-tumor activity of induced anti-suppressor T-cells and other effector cells. Recent advances in the treatment of melanoma focus on targeting mechanisms of tumor immunosuppression, including cytotoxic T lymphocyte-associated antigen 4 (CTLA4) and programmed death-1 receptor (PD1). This review summarizes fundamental concepts and recent advances in our understanding and treatment of melanoma. Ongoing development of novel therapeutic approaches concurrent with optimization of existing therapies and identification of effective combination treatment regimens continue to hold much promise in the field of melanoma research.

CYTOKINES

A number of cytokines, including Interleukin-2 (IL-2), Interferon-a (IFN-α), alone or in combinations with IL-2, IL-12 and others have been tried with various degrees of success in the therapy of melanoma (Table 1).

Interleukin-2 (IL-2)

The biological effects of IL-2 are complex. Relevant for cancer therapy is the enhancement of CTL and NK-cell lysis. In response to IL-2 stimulation, a mixture of NK and $CD8^+$ cells acquire cytolytic properties, which lead to tumor cell killing in vitro, even in the absence of HLA-class I restriction. Complete responses (CR) were produced in 6% and partial responses (PR) in 16% to 20% of patients with metastatic disease that failed standard therapy that were treated with a high-dose (HD) regimen of 600,000 to 720,000 IU/kg i.v.,

repeated every 8 hours for 8 to 14 doses per cycle with two cycles spread by a week considered a treatment course.[5,17] However, many patients are not able to tolerate more than 8 doses per cycle of treatment, with the second cycle typically consisting of fewer doses than the first. Although initial murine studies supported improved results with the addition of LAK cells to HD IL-2 therapy, subsequent clinical data in patients with metastatic disease showed an equivalent efficiency with the HD IL-2 alone.[18,19] With a median response duration of 8.9 months and 44% of responders being alive at 6 years, HD IL-2 offered the possibility of cure for a small fraction of patients.[20] An analysis of 374 metastatic melanoma patients receiving high-dose IL-2 suggested that patients having only cutaneous metastasis had a higher response as compared to however patients with diseases at other sites. Additionally, in the same analysis, it was found that development of vitiligo and thyroid dysfunction was associated with response.[21] An elegant study by Sabatino et al[22] used a multiplex antibody-targeted protein array platform to identify pretreatment elevation in VEGF and fibronectin as being indicators of poor response to IL-2 therapy.

The toxicity of IL-2 therapy prompted studies assessing feasibility of lowering the doses of IL-2. While this results in reduced adverse effects, it is associated with an inferior RR (frequently under 5%), duration and quality of responses.[23] A frequently employed continuous infusion of IL-2 of 18 MU/m^2/d for 5 days is characterized by a similar toxicity and response rate as HD IL-2, but a shorter duration of response.[6] The current consensus is that although the high dose regimens are currently approved by the FDA, the issue of optimum dose appears to still be unresolved, primarily due to lack of randomized data controlled for prognostic factors across institutions.

Interferon-α (IFN-α), IL-2$^+$IFN-α Combinations

IFN-α exerts anticancer activities through numerous mechanisms, including direct antiproliferative/apoptotic effects,[24] increasing immunogenicity of tumors,[25] suppression of angiogenesis,[26,27] as well as modulating the innate and adaptive immune response.[28] Response rates of 14% with rhIFN-α2a and up to 23% with IFN-α2b were obtained in Phase II trials in metastatic melanoma using various schedules and dosages.[29] Accumulating clinical experience showed that responses to IFN-α can be delayed for a couple of months, and continuous treatment is more effective. One potential interpretation of these findings is that IFN-α stimulates clonal expansion of cytotoxic T cells that naturally are subjected to tumor-mediated immune suppression.[30]

The rationale for combining IL-2 with IFN-α resides in the up-regulation by the latter of HLA and tumor-associated antigen (TAA) expression on tumor cells, which may enhance the T-cell lysis induced by IL-2.[31] However, clinical trials have not confirmed the anticipated benefits of combination therapy over HD IL-2 alone.[7,32] Similarly, trials combining IL-2 with granulocyte-macrophage colony stimulating factor (GM-CSF), tumor necrosis factor (TNF), IFN or IL-4 did not result in any meaningful clinical improvement as reviewed in ref.23

Other Interleukins and Cytokines

In metastatic melanoma patients, use of IL-12 was associated with limited activity in phase I trials, with one transient CR among 12 patients treated at the maximum dose level.[33] Only minor responses were seen in 30% of cases in another study.[34] The antitumor effects of rhIL-12 appear to be correlated with serum IFN-γ levels, and clinical benefits appear to correlate with maintaining its secretion after four weeks of treatment, as demonstrated in a trial assessing the combination of IL-12 with low-dose IL-2.[35] Furthermore, recombinant cytokines such as IFN-γ,IL-1,IL-4,IL-6,TNF-α and IL-18 have been experimented with modest results in the treatment of metastatic melanoma.[36–38]

ADOPTIVE IMMUNOTHERAPY

The historical development of cellular immunotherapy targeted activation of the innate effector immune cells: macrophages, NK, and LAK. A great enthusiasm for these approaches emerged as successful results were obtained in animal models, showing an enhancement of IL-2 efficacy when combined with LAK cells derived from peripheral lymphocytes. However, clinical trials using IL-2 and LAK cells did not confirm a substantial advantage compared to high-dose IL-2 alone.[18,19] In contrast, it appeared TILs had more promising activity. For example, autologous TILs plus HD bolus IL-2, with or without administration of cyclophosphamide, produced a 33% RR. Furthermore, the use of TILs generated from subcutaneous tumor deposits showed a higher RR (49%), when compared with the use of those originating from lymph nodes (17%) (P=0.006).[39] Mechanistically it appears that IL-2 co-administration provides a fertile ground for TIL-expansion and/or survival in vivo.[40] A note to consider are recent studies demonstrating expansion of $CD4^+$ $CD25^+$ T regulatory cells subsequent to administration of systemic IL-2.[41] It may be therefore be worth considering Treg depletion as part of TIL^+ IL-2 approaches.

A significant advancement was recognition that TIL functional parameters, such as their ability to recognize and lyse tumor targets, may correlate clinical with responses.[42,43] In addition, in vivo persistence and telomere length of transferred TILs has also been correlated by some authors with the antitumor response.[44,45] With the notion of TIL having activity that correlates with outcome, the next question would be how to improve on that activity? One innovative approach involved utilizing the concept of homeostatic expansion. Essentially, lymphocytes are regulated by a balance between stromal cell generated IL-7 and IL-15 and ability of the lymphocytes to uptake these cytokines. When levels of lymphocytes drop systemically, there is an excess IL-7 and IL-15 which results in activation of low-affinity receptors on lymphocytes. This causes loss of costimulatory requirement and allows previously antigen-nonreactive lymphocytes to because antigen reactive.[46] Supporting this notion are numerous animal studies and human observations reviewed by Marleau et al.[47] Translating this concept into clinical practice, lymphodepletion was performed by cyclophosphamide and fludarabine followed by administration of highly-selected, antigen-specific TIL. Six PRs and 4 mixed responses were achieved in 13 refractory melanoma patients.[48] These results were confirmed in a larger study, where a similar regimen produced a 50% RR (8% CRs).[49] The rationale for prior administration of lymphodepleting agents is to eliminate T regulatory (Treg) cells,[50] as well as remove cytokine sinks,[46] thus allowing for generation of costimulatory-independent tumor-targeting responses.

Intensification of the lymphodepleting regimen by using cyclophosphamide plus fludarabine along with 2 to 12 Gy of TBI before autologous TIL administration resulted in an increase in the serum levels of lymphocyte homeostatic cytokines IL-7 and IL-15. This immune activation was accompanied by clinical response rates of 52% and 72%, respectively, for the two radiation dose intensities.[51] The anti-tumor responses were correlated with the number of $CD8^+CD27^+$ cells infused, as well as with their mean telomere length and their persistence in circulation at one month after administration.[52] The 28% rate of CRs achieved through adoptive immunotherapy after maximum lymphodepletion sets a new standard in immunotherapy, considerably superior to the results of IL-2 alone, vaccination, or a combination of the two.

Other recent advances in adoptive cell transfer involve the use of autologous T cells engineered to express T-cell receptors with specificities for various tumor associated antigens or to secrete cytokines. Autologous T cells transduced ex vivo with anti-MART-1-TCR genes persisted for a prolonged time in vivo and led to sustained objective regression in two patients with metastatic melanoma. However, the overall RR (13%) was still inferior

to those produced by the infusion of autologous TILs (50%).[53] Adoptive cell transfer of TILs engineered to produce IL-2 demonstrated high IL-2 production and prolonged survival upon IL-2 withdrawal in vitro. In vivo, however, IL-2 transduced TILs elicited the same RR and persistence as unmanipulated TILs. Telomere shortening as a result of the in vitro expansion was cited as a possible cause of mediocre results.[54]

BIOCHEMOTHERAPY

Achievement of durable responses with biological agents, and the possibility to complement the higher response rate of chemotherapy by prolonged duration of remissions led to development of biochemotherapy. Among potential advantages of this combination is an enhancement of immune recognition and cellular effector activity triggered by IL-2 in the setting of tumor cellular disruption and antigen release induced by chemotherapy agents. Furthermore, administration of chemotherapy first may enhance immunity and decrease the tumoral mass, therefore augmenting the effectiveness of response. Although a clear improvement in response rate (40% to 60%) resulted from the use of biochemotherapy, several randomized phase III studies produced mixed results on the duration of survival, and two recent meta-analyses did not reveal any improvement in OS.[55,56] A meta-analysis of clinical trials employing various time frames between the administration of chemotherapy and biologics showed that, as the time-frame between chemo and bio components increases, the OS, survival of CRs and PRs appear to increase, but the effect is only present for the chemo-first combination. It appears possible that the interaction between components of biochemotherapy results in a double effect: an increase in the immediate response, correlated with production of nitric oxide which acts synergistically with chemotherapy in producing tumor cell killing, and an increase in survival, correlated with macrophage activation, as measured by neopterin levels.[57]

VACCINES

The goal of vaccination is to induce immune recognition against antigens expressed by tumors.[58] A great variety of approaches have been utilized over the past 35 years. Despite occasional tumor responses and even suggestions of improved survival, no consistent results were obtained with the early allogeneic or autologous whole cell vaccines, cell lysates, and shed antigen preparations.[59]

Discoveries in characterization of tumor antigens and biology of immune reactions have shifted the focus towards construction of vaccines based on specific antigens, such as peptides, or enhancing their presentation through the use of dendritic cells. There are a limited number of completed phase III trials, and although some reported marginal benefit, no benefit, or even harm, this research has nevertheless improved our understanding of tumor immunobiology, and shed important information on the future direction of investigation.[60] One concrete benefit of these studies was the appreciation that development of autoimmune manifestations, like vitiligo, may serve as a clinical marker for responsiveness to therapy and improved survival.[21,61] Interestingly, as discussed in the situation of high dose IL-2 therapy, similar autoimmune-like manifestations appear in some situations to be a common feature of successful immunotherapy.[62]

WHOLE CELL AND LYSED CELL VACCINES

Limited immune responses can be elicited using allogeneic and autologous tumor vaccines, and the clinical benefit of these preparations has remained modest. In early clinical trials, vaccines prepared from whole tumor cells were associated with limited activity.[63] Nevertheless, continued exploration of such multivalent approaches has proceeded. In early phase II trials, vaccination with Melacine was associated with up to 10% clinical responses,

while 74% of patients had either an objective remission or disease stabilization when used in conjunction with IFN-α2b.[64] However, subsequent randomized controlled clinical trials comparing Melacine and IFN-α2b versus IFN-α2b alone found no significant difference in relapse-free survival (RFS) or overall survival (OS) between the two treatment arms. Median OS time exceeded 84 months in patients receiving melacine plus IFN-α2b, and was 83 months in IFN-α2b alone (P=0.56, 95% CI, 60 months to not reached).[65] One possible explanation for the poor results of such polyvalent approaches may be related to various immune suppressants and angiogenic agents found in the vaccine mixture. An interesting approach to overcome this may be the use of xenogeneic vaccines. For example, it has been reported that self-tolerance to angiogeneic agents such as VEGF, FGF, and EGF may be broken by administration of xenogeneic homologues.[66] Administration of xenogeneic tumor antigens has been performed clinically with promising results;[67] however, to our knowledge this approach has not been evaluated in a polyvalent setting.

Peptide Vaccines

Poor efficacy of complex tumor cell-derived polyvalent vaccines has prompted interest in more defined antigens. Although highly selective approaches have the advantage that: (a) immunogenic epitopes can be selective for; and (b) epitopes can be modulated (eg, altered peptide ligands) to artificially increase immunogenicity. The first defined human cancer-testes antigen, MAGE-1, was characterized in 1991.[3] Since then a long and growing list of potential targets for cancer immunotherapy has emerged. In this context, metastatic melanoma served as the first model to test immune responses to peptide vaccines.

Spontaneous T-cell responses to the native gp100 antigen have been noted in patients with metastatic melanoma who experienced tumor regression following adoptive therapy with TIL and IL-2. Rosenberg et al[68] utilized a cancer vaccine consisting of immunodominant peptides derived from the gp100 melanoma-associated antigen, administered with a synthetic peptide designed to increase binding to HLA-A2 molecules. Of patients receiving the vaccine plus IL-2, 42% had an objective response. However, three different schedules of high-dose IL-2, administered with the 209-2M peptide did not produce better activity than expected with IL-2 alone (OR 14.5%, CR 8%).[69] Vaccination with the synthetic modified gp100: 209 to 217 (210 M) peptide, created by altering the anchoring amino acid (methionine in place of threonine at position 2), produced an increase in specific $CD8^+$ T cells after vaccination in 28 of 29 patients, of whom 77% remained disease-free at 23 months.[70] Another approach, consisting of addition of tetanus peptide as a nonspecific helper epitope resulted in Th-cell responses in 79% of patients and an OS of 75% at 4.7 years of follow-up, which compared favorably with the expected survival.[71] However, clinical responses are not always correlating with the immune activation, as exemplified by a study involving vaccination with the HLA-A*0201-restricted Mart-1/Melan-A(27 to 35) and incomplete Freund's adjuvant (IFA), where no clinical activity was observed, despite a 94% rate of CTL-specific responses.[72] Conversely, no evidence of enhancement of the systemic immune response could be documented in nine patients immunized with the peptide MAGE-A12: 170 to 178 administered in IFA, although one patient had an ongoing PR.[73]

A paradoxical dichotomy between elicitation of immune responses and clinical activity has also been seen with the modified gp100 epitope g209-2M. While 91% of patients showed successful immunization, no clinical responses were observed. Moreover, only 16% of patients treated with $IL2^+$g209-2M vaccination developed immune reactivity, whereas 13 of 31 (42%) demonstrated clinical responses.[74] An explanation for this phenomenon can reside in trafficking of the vaccine-specific T cells to the tumor site, although it is possible that the discrepancy originated in a differential susceptibility of CTLs to in vitro sensitization.[75]

A recent breakthrough was seen in a multicenter, randomized, prospective trial compared HD IL-2 alone with IL-2 and gp100 209 to 217 peptide in 185 stage III/IV patients expressing HLA0201. The response rate was significantly higher in the combination treated (22.1%) as compared to the IL-2 alone treated patients (9.7%). A significant improvement was seen also for the progression free survival 2.9 months (1.7 to 4.5) compared to 1.6 months (1.5 to 1.8), respectively.[76] This appears to be the first description of clinical benefit in metastatic melanoma using vaccination.

In the E1696 trial assessing multiepitope (MART-1, gp-100, and tyrosinase) vaccine alone, in combination with either GM-CSF, IFN-α2b, or in combination with both, of 115 patients who were analyzed, median survival of patients exhibiting immune response to at least one of the antigens was prolonged as compared to those lacking induction of immunity (21.3 mo vs. 13.4 mo; P=0.046). There was no difference in terms of immunity between patients receiving the vaccine alone or in combination with cytokines.[77]

IL-12$^+$ Peptide Vaccines

Murine studies have shown that IL-12 promotes potent antitumor immunization when co-administered with peptides loaded onto other class I MHC$^+$ cells, thus potentially bypassing the need to use DCs. In a Phase I clinical trial, patients with metastatic melanoma received autologous PBMCs pulsed with a MAGE-3 or Melan-A peptides and co-administered with various doses of rhIL-12. Antigen-specific CD8$^+$ T-cell responses were demonstrated in six out of eight patients having sustained clinical responses.[78] Another study found no clear dose-dependent effect of rhIL-12 on the responses to Melan-A and influenza matrix peptides administered intradermally. However, rhIL-12 was well tolerated at doses of 10 to 100 ng/kg, and 3/24 melanoma patients demonstrated clinical activity.[79]

GM-CSF$^+$ Peptide Vaccines

Use of GM-CSF as a vaccine adjuvant is appealing because of its role as one of the primary growth and maturation factors for DCs. A randomized trial compared three different adjuvants in HLA*A0201+ patients with stage III or IV melanoma immunized with tyrosinase and gp100 peptides. 44% and 50% of patients immunized using QS-21 (a purified saponin) and GM-CSF, respectively, developed increased frequencies of CD8$^+$ T cells against tyrosinase 370D peptide, compared with 0 of 9 patients immunized using IFA (P=0.045).[80] Immunization against 3 HLA-A2-binding peptides of the cancer-testis antigen NY-ESO-1, a strongly immunogenic tumor antigen, followed by administration of GM-CSF resulted in 4 of 7 NY-ESO-1 antibody-negative patients developing a specific CD8$^+$ T-cell response, and was associated with stabilization or regression of metastases in 5 of 7 cases.[81] There may be some rationale for restricting dose and site of GM-CSF administration. Tumor-secreted GM-CSF has been speculated to actually play an immune suppressive role through increasing numbers of CD34 expressing myeloid suppressor cells.[82,83] In addition, some tumor vaccination studies have demonstrated enhanced tumor growth and immune suppression depending on dose of GM-CSF administered.[84]

Recombinant (Gene-modified), Viral, and Plasmid Vaccines

Gene modified tumor vaccines are commonly designed using autologous melanoma cells that have been transfected with an immunostimulatory gene, such as the expression of an immune enhancing cytokine. Immune stimulation that translates into antitumor activity was demonstrated in a B16 melanoma model, in which irradiated tumor cells expressing murine GM-CSF (and to a lesser extend cells expressing IL-4 and 6) stimulated long-lasting, and specific anti-tumor immunity, requiring both CD4$^+$ and CD8$^+$ cells.[85] Stimulation of immunity may have been the result of enhanced antigen presentation by local dendritic cells whose maturation was induced by tumor secreted GMCSF, although this was not formally

demonstrated. In a Phase I trial investigating autologous melanoma cells engineered to secrete human GM-CSF, induction of tumor-specific responses were observed. Metastatic lesions were densely infiltrated with T lymphocytes and showed extensive destruction in 11 of 16 patients, which was associated with anti-melanoma CTL and antibody responses. One PR, one mixed response, and three minor responses were achieved, and three patients remained disease-free at 36, 36, and 20 months.[86] Another approach used mixtures of autologous and allogeneic irradiated melanoma cells secreting IL-6 and sIL6-R. This evoked immune activation and promising clinical results (22% CR^+PR, 32% SD).[87] Several factors need to be contemplated when deciding appropriate choice of genes for tumor transfection, for example, ability to stimulate DC maturation, ability to increase immunogenicity of tumor directly, and possibility of synergizing with existing immune responses. Interestingly, in some situations, transfection of tumors with agents that are considered to be immune suppressive, such as interleukin-10, actually evoke anti-tumor immunity (reviewed in[88]).

An alternative approach to immunizing with gene-modified tumors is to locally transfect muscle tissue using recombinant adenovirus vector encoding tumor antigens. For example, intramuscular delivery of gp100 or MART-1 using this approach, either alone or followed by IL-2, resulted in one CR in 16 patients pertaining to the group receiving the recombinant adenovirus MART-1 alone.[89] Detection of high titers of neutralizing antibodies to the adenoviral vector may explain the relatively low efficacy of this approach. In any case, the low number of patients in this study does not rule out the possibility of expanding on this approach given similar low rates of responses to other immunotherapeutic approaches.

Given that the tumor already expresses a wealth of tumor antigens, studies have been conducted to enhance tumor immunity by inducing a potent local inflammatory response in the tumor site itself. One example of this is Allovectin-7, a plasmid DNA encoding HLA-B7 and beta-2 microglobulin.[90] When injected into melanoma lesions, it resulted in up to 15% rate of PRs, which occurred even at remote sites. An OS of 21.3 months was achieved with high doses of the bicistronic vaccine, and corresponded to a 12.7 months median duration of responses.[91]

Dendritic Cell (DCs) Vaccines

The $CD8^+$ cytotoxic T-cell compartment, which is believed to possess major anti-tumor effector function, usually is poorly activated in cancer patients (Fig. 1). One of the postulated mechanisms is the inadequate tumor antigen presentation by DCs. This may be explained by a variety of factors including tumor secretion of factors inhibiting DC function,[92,93] as well as indirect inhibition of DC function by Treg cells,[94] which are increased in melanoma patients.[95] Therefore, use of de novo generated dendritic cells has been part of the new strategies to enhance CTL responses (Table 2). The goal of dendritic cell vaccines is to induce a Th1 immune response and to activate CTLs, in order to facilitate tumor elimination.

DCs are capable of processing and presenting peptides derived from tumor protein antigens to $CD4^+$ and $CD8^+$ T cells, and of regulating the activity of natural killer (NK) cells. Matured from $CD14^+$ precursors, ex-vivo DCs are loaded with antigens in the form of whole proteins, tumor lysates, peptides, necrotic and apoptotic bodies, or messenger RNA. Specific epitopes can be pulsed in the form of synthetic HLA-binding peptides, or DNA and RNA sequences carried by viral vectors. Immature DCs are able to process antigens, but it is the mature DCs that are able to fully stimulate T cells by upregulating cytokine secretion, adhesion and costimulatory molecules (Fig. 1).[113–115] Animal experiments with primarily mature DCs consistently showed inducement of tumor-specific CTL responses, and occasional regression of metastases.

In a landmark study using mature DCs pulsed with Mage-3A1 peptide and a recall antigen (tetanus toxoid or tuberculin), Thurner et al[116] demonstrated significant expansion of CTL in 8/11 patients with advanced stage IV disease, and objective responses in 6/11 patients. In another trial, 5 clinical responses occurred in 16 metastatic melanoma patients treated with tyrosinase 370D and gp100 210M peptides restricted to HLA class I A*0201. The peptides were modified to increase immunogenicity by altering one aminoacid from the wild type, and were pulsed into DC derived by incubation of plastic-adherent peripheral blood mononuclear cells (PBMC) with IL-4 and GMCSF. Among five patients having a CTL response to gp100 or tyrosinase, four were clinically stable or had tumor regression. Immune responses to gp100 or tyrosinase were demonstrated by gamma intereferon ELISA assay in 31% of patients.[98] Vaccination with mature DCs loaded with three tumor lysates (M44, SK-MEL 28 and COLO 829), tested in a phase II trial evaluating patients with low volume or in-transit melanoma, produced two clinical responses and 4 stable disease in 33 metastatic melanoma patients. Ten patients showed evidence of enhanced anti-TAA tumor specific $CD8^+$ T cells.[109] A correlation between the enhanced immune response and survival was seen with DCs pulsed with MART1, tyrosinase, MAGE3, gp100 and influenza matrix peptides, along with keyhole limpet hemocyan (KLH). Six out of seven patients with immunity to two or fewer antigens progressed, in contrast to only 1/10 patients with immunity to more than 2 antigens.[117]

NK T-cell activation has also been demonstrated in phase I/II testing of vaccination of patients with metastatic melanoma with intermediate-maturity DCs engineered to express MART-1 through an adenoviral vector. $CD8^+$ and/or $CD4^+$ MART-1-specific T-cell responses were observed in 6/11 and 2/4, respectively, of the patients being evaluated.[118] Objective responses were evident in 5 of 16 patients treated with immature DCs pulsed with tumor lysate or a cocktail of peptides in the presence of GM-CSF and IL-4. In this study, elicitation of delayed type hypersensitivity (DTH) reactions was seen in 11 patients, and recruitment of peptide-specific CTLs was demonstrated.[96]

In a review of 32 clinical trials on dendritic cell vaccines, Engell-Noerregaard et al[119] found that clinical response (defined as CR, PR, or SD) was significantly correlated with the use of peptide antigens, use of helper antigen or adjuvant, and induction of tumor antigen-specific T cells. The majority of studies, however, show that despite repeated T-cell activation with antigen-loaded mature DCs, expansion of tumor-antigen-specific immune responses is often transient, and only rarely produces stable disease or regression of tumor metastases.[97,120,121] Although significant expansions of specific $CD8^+$ cytotoxic T lymphocytes have been demonstrated for Mage 3A1 in over 70% of patients,[116] clinical results with peptide-pulsed DCs remain disappointing, indicating a failure of immunological data to translate into clinical success. Schadendorf et al[122] actually reported no benefit of peptide-pulsed DC in comparison to dacarbazine (DTIC) treatment. Regulatory T cells (Tregs) may produce a potent down-regulation of anti-tumor responses that counteract the protection conferred by dendritic cell vaccines (Fig. 1). Tumors can also mediate DC suppression, and potential molecular targets are now being identified, including p44/42 MAPK, which is hyperactivated in melanoma, and its upstream activator MEK1/2. Blockade of the MEK1/2-p44/42 axis has been shown to increase IL-12 production and enhance Th1 immune responses.[123]

Heat-shock Proteins (HSPs)

HSPs have important immune functions: they chaperon intracellular antigenic peptides, induce maturation of DC, and activate NK, $CD4^+$ and $CD8^+$ T cells. Two durable CRs (559+ and 703+ d) and 3 SDs were produced in 28 metastatic melanoma patients vaccinated with autologous, tumor-derived heat shock protein gp96-peptide complexes (HSPPC-96, Oncophage). The ELISPOT assay showed an increased melanoma-specific T-cell activity in

11 of 23 patients, which correlated with clinical responses,[124] but no further improvement was obtained by modulating the immune reaction with GM-CSF and IFN-α.[125]

More recently, however, a phase III trial enrolling 322 patients with metastatic melanoma found no difference in OS between the HSP vaccine, vitespan (an autologous tumor-derived heat shock protein gp96 peptide complex vaccine), and physician's choice of treatment, including dacarbazine, temozolomide, interleukin-2, or complete tumor resection.[126]

Given that mechanistically, HSP-based vaccine approaches appear to function through increasing immunogenicity of the bound peptides, it may be useful to view this therapy as a "sophisticated polyvalent lysate". This would suggest the need for studies to calibrate the degree of immunogenicity between the various HSP-antigen fractions, as a possible next step in improving this therapeutic.

STRATEGIES OF IMMUNE ENHANCEMENT

Despite a highly antigenic load resultant from structural and acquired genetic instability, melanoma and other tumors are able to avoid immune inhibitory influences. Tumor induced mechanisms of immune escape are diverse, and pose limitations to the effective use of immunotherapy. Such immune downregulatory influences probably explain the failure of preclinical models to translate in clinical efficacy when applied in vivo. Important mechanisms of immune escape include downregulation of certain components of the antigen processing machinery by tumors, including β2 microglobulin, and transport associated with antigen processing (TAP)-1 and TAP-2 peptide transporters, which are critical to MHC class I antigen presentation pathways.[127–129]

Other postulated mechanisms for tumor escape include production of immunosuppressive factors such as the Fas ligand, TGF-β, IL-6, IL-10, PGE2, VEGF, or suppression of co-stimulatory molecules such as CD40, CD80, and CD 86, although the disturbed cellular apoptotic and proliferation cellular pathways may create myriads of immune inhibitory effects. Blocking such cellular pathways, which are disturbed as a result of genetic alterations during tumorigenesis, tumor progression, or tumoral inhibition of the effector T cells, by using small-molecule tyrosine kinase inhibitors or blocking antibodies, can result in immune augmentation and enhancement of vaccine-triggered anti-tumor immunity. In fact, a proof of principle for overcoming tumor resistance is the effectiveness of IFN in enhancing response to tumor vaccines.[130] Other methods of increasing tumor immunogenicity include treatment with histone deacetylase inhibitors such as valproic acid. For example, Khan et al[131] demonstrated increased expression of antigen processing machinery (TAP1, TAP2, LMP2, LMP7, Tapasin), costimulatory molecules (CD40, CD80) and MHC class I on melanoma cells after treatment with a variety of histone deacetylase inhibitors.

Another method of tumor immune evasion involves secretion of microvesicles termed "exosomes", that have been demonstrated to contain tumor antigens on MHC I,[132,133] and a variety of immune suppressive molecules such as HLA-G,[134] FasL,[135] as well as factors associated with inhibition of DC maturation.[136] The combination of antigen with inhibitory signaling suggests the possibility that exosomes may mediate antigen-specific immune suppression. Possible mechanisms for clearing exosomes have been proposed such as hollow-fiber dialysis.[137] Perhaps a more exotic means of reversing immune suppression is vaccination with xenogeneic exosomes, which would induce induce immune responses towards tumor antigens and also associated immune suppressive molecules.

Immunomodulatory Monoclonal Antibodies

As the understanding of the dynamic and complex interaction between the immune system and tumors improves, new immunotherapies targeting critical regulatory elements of the immune system can be developed in order to provide treatments with greater specificity and better safety profiles. This includes the recent development of anti-CTLA-4 monoclonal antibodies, Toll-like receptor (TLR) agonists, CD40 agonists, and anti-ganglioside monoclonal antibodies. A family of receptors on T cells serves as a natural braking mechanism for T-cell activation, functioning to reestablish homeostasis following an immune response, and to maintain peripheral self-tolerance. This includes CTLA4 and PD1.[138]

CTLA-4 expression on T cells outcompetes CD28 for binding to CD80 (B7–1) and CD 86 (B7–2), resulting in suppression of T-cell activation and depending on the model assessed, modulation of cytokine production.[139] In addition, the high affinity binding of CTLA-4 to CD80/86 stimulates the antigen presenting cell to generate high concentrations of the immune suppressive enzyme indola-mine 2,3 deoxygenase.[140] This understanding provided the rationale for development of anti-CTLA4 monoclonal antibodies that could inhibit interaction between B7 and CTLA4, thus releasing the “brakes” against T-cell activation that was hypothesized to enhance antitumor immune response. This was confirmed in animal studies showing that CTLA4 blockade enhanced antitumor T-cell function and inhibited tumor recurrence in murine prostate cancer and melanoma models.[141–143]

Ipilimumab

Anti-CTLA4 monotherapy with ipilimumab has produced clinical response rates in 7% to 15% of patients with metastatic melanoma.[144] In one study, the CTLA-4 antibody ipilimumab (formerly MDX-010), produced objective responses in 17% of patients, including 3 patients who demonstrated complete responses (ongoing at 23+, 52+, and 53+ mo).[145] To enhance antitumor response, CTLA4 blockade has been investigated in various dosing regimens as well as in combination with cancer vaccines, standard therapies such as chemotherapy, and IL-2 administration (Table 3). Ipilimumab administered in conjunction with a gp-100 peptide was able to induce a 21% response rate (14% CR).[146] The same antibody alone or in conjunction with dacarbazine produced durable responses in patients with melanoma, some lasting over 1 year.[147] Combination therapy with ipilimumab and IL-2 showed an objective response rate of 22% in patients with metastatic melanoma. An additive, but not synergistic, effect between IL-2 and ipilimumab was observed.[148]

Recent studies have also noted cases of late onset objective response among patients who previously experienced stable disease or disease progression.[150,154] This development prompted a suggestion to continue observation or treatment in patients with initial progressive disease or stable disease. Objective antitumor response is often associated with immune related adverse events, most commonly involving the skin and gastrointestinal tract.[145,155] For example, in the study of Sanderson et al[155] involving a multiple peptide vaccine in conjunction with CTLA-4 blockage, only 37.5% of patients with autoimmune effects experienced a relapse, compared to 81.8% of those without autoimmunity.

Other studies suggest that prior cytokine therapy may pose a negative prognostic factor for survival in patients later receiving treatment with anti-CTLA-4 monoclonal antibodies, although differing results have been reported between IFN and IL-2.[145,156]

Tremelimumab

Tremelimumab (CP-675206), a fully human immunoglobulin G-2 anti-CTLA-4 mAb, was shown in phase I and II studies to produce durable objective responses in patients with

melanoma, ranging from 7% to 14% at dose levels between 0.01 to 15 mg/kg.[151,152] Subsequently, a phase III randomized clinical trial was initiated to compare tremelimumab to standard chemotherapy (dacarbazine or temozolomide) in patients with advanced relapsed or refractory melanoma. Among 655 previously untreated patients, no statistically significant difference in overall survival was observed between the two treatment arms (median OS 11.8 and 10.7 for tremelimumab and chemotherapy, respectively, HR 1.04), however differences in long-term survival are still unknown.[152] In the setting of advanced Stage IV melanoma, CTLA-4 blockade with tremilimumab demonstrated restoration of effector and memory $CD4^+$ and $CD8^+$ T cells, and induction of transient T-cell resistance to Treg-mediated suppression, which was correlated with clinical outcome.[157]

Increased recognition that common genetic variation in drug targets could affect clinical response to CTLA-4 blockade therapy has led to the recent incorporation of pharmacogenetic analysis to evaluate common polymorphisms in the CTLA4 gene and their influence on the response to tremelimumab. Correlation of patient genotype with clinical response has so far demonstrated inconsistent trends[158,159] and further studies are necessary.

Anti-PD-1 mAbs

PD-1 is another inhibitory molecule found on the surface of T cells that is associated with tolerance induction upon binding to its ligands PD-L1 and PD-L2. PD-1 is also expressed by several tumors, including melanoma, where it inhibits antitumor responses and mediates tumor evasion.[160,161] Preclinical studies demonstrate that monoclonal antibodies against PD-1 improve immune functions of tumor-specific T cells, enhance cytokine production, and increase tumor lysis.[162] Phase I trials of MDX-1106/ONO-4538, a fully human anti-PD1 blocking antibody, are ongoing.

CD40 Agonist mAbs

The CD40 cell-surface costimulatory molecule is naturally expressed on dendritic cells, B lymphocytes, monocytes, even solid tumors, and has a broad range of functions. CD40 is upregulated on activated DCs. The engagement with its natural ligand CD154 (CD40L), primarily expressed on activated CD4+ T cells, triggers cytokine secretion and enhanced expression of costimulatory molecules required for efficient T-cell activation.[163] It has been demonstrated in murine models that in vivo delivery of CD40-activating antibodies overcomes the immunosuppressive mechanism of tumors, increases T-cell activation, and enhances antitumor immunity, leading to regression of established tumors.[164] Other preclinical studies testing CP-870,893, a recombinant human agonist monoclonal antibody against CD40, have also demonstrated enhanced in vitro anti-tumour T-cell responses, evidenced by a significant expansion of IL-2 and IFN-γ-producing cells, as assessed by ELISpot assay.[165] In early phase I trials, CP-870,893 was well tolerated and was able to produce objective tumor response in 27% of melanoma patients.[166]

Activation of Innate Immunity (TLR Agonists)

Newer strategies to overcome tumor-induced immune suppression involve attempts to improve presentation of tumor-associated antigens through enhanced expression of co-stimulatory molecules on the surface of DCs.

Toll receptors (TLR) are signaling molecules that recognize conserved molecular patterns on common pathogens, which are able to influence the activity of DCs and induce T-cell responses. TLR-stimulating ligands are being evaluated for their potential to enhance DC activation and heighten antitumor immune responses. Attention has largely focused on TLR-9 agonists, although TLR7/8 ligands also show promise (Table 4).

The use of PF-3512676 (formerly CpG 7909), a synthetic deoxycytidyl-deoxyguanosine oligonucleotide which activates TLR-9, has been associated with a 10% PR and with one response lasting for 13+ months.[168] Clinical response has been associated with the stimulation of NK-cell cytotoxicity, as well as with direct effects on increased activation of DCs, which subsequently induce potent innate immune responses via proinflammatory cytokine secretion, activation of other immune effectors (eg, NK-cells), and increased antigen presentation.[170]

TLR7/8 agonists demonstrated in preclinical studies an ability to markedly enhance the antitumor responses though diverse mechanisms involving maturation, activation, and/or migration of critical effector cells, including dendritic cells, B cells, T cells, NK cells, and mast cells. Dendritic cells have been shown to respond to TLR 7/8 agonists by increased secretion of IFN-α, IL-12, TNF-α, as well as upregulation of costimulatory molecules such as CD80 and CD86, increased polarization towards Th1-type responses, and enhanced tumor lysis.[172–174] B-cells, upon treatment with TLR7/8 agonists, are stimulated to increase production of cytokines and antibodies, as well as to upregulate CD80/86 which is essential for T-cell activation. These agonists also act directly on T cells by increased production of Th1-polarizing cytokine.[175] The effects described above may render tumor cells more immunogenic and more susceptible to chemotherapy-induced tumor lysis. Inhibition of angiogenesis and promotion of apoptosis have also been associated with TLR7/8 agonists.[176]

Imiquimod, a synthetic TLR7 agonist, when utilized to activate DCs in a group of patients vaccinated with influenza, Melan-A, tyrosinase and NY-ESO peptides, elicited $CD8^+$ T-cell responses in 5/8 patients, and one patient in 12 achieved a PR.[177] Other cases have been reported of complete clinical clearance of locally metastatic melanoma when treated with topical 5% imiquimod[178] alone, or in combination with tazarotene cream.[179] These responses are likely mediated by several effects on dendritic cells, ranging from increased recruitment to the skin, enhanced migration to lymph nodes upon antigen uptake,[180] and functional maturation,[181] as demonstrated in preclinical studies. Alternatively, imiquimod has been demonstrated to directly increase immunogenic molecule expression of melanoma cells, as well as to induce apoptosis, thus providing an ample supply of local immunogenic proteins.[182]

TLR agonists are able to produce key alterations in the tumor microenvironment, but despite successful induction of Th1 antibody responses and of tumor antigen-specific $CD8^+$ T cells, the development of clinically meaningful responses and improved survival have yet to be seen in patients with melanoma being treated with stand-alone TLR agonists. The potential use of TLR agonists as adjuvants in cancer vaccine or adoptive immunotherapy approaches are under ongoing investigation.

Depletion of Immunosuppressive Cells (Treg Depletion)

Regulatory T cells (Tregs) are immuosuppressive elements that, under normal physiological conditions, help modulate the immune response to prevent autoimmunity. In cancer, however, Tregs suppress antitumor responses of both $CD4^+$ and $CD8^+$ T cells, and their number in patients with Stage IV melanoma has been found to correlate inversely with survival (P=0.004).[183]

Two distinct populations of Tregs are recognized: naturally occurring $CD4^+/CD25^+$ Tregs (nTregs) which arise from the thymus with constitutive immunosuppressive function, and induced Tregs (iTregs) comprised of T cells that acquire an immunosuppressive function only under appropriate conditions, in the setting of an immune response.[184] The most widely studied are $CD4^+/CD25^+$ Tregs. CD25 represents the alpha subunit of the IL-2 receptor,

which is also highly expressed by activated T cells, thus making it a nonspecific Treg marker. The forkhead transcription factor (Foxp3) is now known to be exclusively expressed in $CD4^+CD25^+$ regulatory T cells, and is required for Treg development. Additionally, transduction of Foxp3 to conventional $CD4^+CD25^-$ T cells was shown to be sufficient to confer suppressor function.[185]

The mechanisms by which Tregs function are not fully understood, but are thought to occur in a cell-cell contact dependent mechanisms,[186] which involves IL-10, TGF-B and other cytokine secretion. A secondary messenger, cyclic adenosine monophosphate (cAMP), known to be a potent inhibitor of proliferation, also appears to be a critical component of nTreg function.[187]

Given their immunosuppressive effects, strategies to promote Treg depletion or inhibition have been evaluated in preclinical and human studies.

IL-21, in particular, may hold promise as an adjuvant therapy to augment response to adoptive T-cell transfer and vaccination approaches. In transfected IL-21-secreting B16 melanoma cell lines, IL-21 was found to delay tumor growth in vivo. The effect is thought to be mediated by an enhanced systemic effector and memory $CD8^+$ T-cell responses, and a decreased accumulation of regulatory $CD4^+FOXP3^+$ T cells within the tumor microenvironment, by as much as 50%, compared to controls.[188] Phase II trials of recombinant human IL-21 (rIL-21) given intravenously or subcutaneously in patients with stage IV melanoma showed acceptable safety profiles and demonstrated clinical responses, with 1 CR and 1 PR among 14 patients treated with intravenous IL-21 and 1 CR and 2 PRs among 23 patients treated with subcutaneous IL-21.[189,190]

Depletion of Foxp3-expressing regulatory T cells has also been demonstrated in preclinical studies using vaccination with Foxp3 mRNA-transfected dendritic cells. Strong induction of Foxp3-specific CTL responses was observed, along with as a preferential depletion of Tregs in the tumor (as opposed to the periphery), which may potentially reduce the risk for autoimmunity.[191]

WP1066, an inhibitor of STAT3 signaling, directly inhibits Tregs in a dose-dependent manner, an effect that was shown to promote enhanced T-cell cytotoxicity against melanoma.[192] STAT3 inhibitors should be tested in combination with other immunotherapies, particularly those known to expand CD4(+) FoxP3(+) Treg populations, such as the anti-CTLA-4 monoclonal antibodies and systemic IL-2.[193–197] Potential synergism may exist, possibly permitting the simultaneous expansion of $CD8^+$ T cells and inhibition of Tregs.

CD25-directed recombinant immuotoxins have also induced a significant reduction of regulatory T-cell populations, as demonstrated in preclinical studies by using RFT5-SMPT-dgA and LMB-2, respectively. Clinical studies, however, have shown only transient, partial reductions of Tregs in patients with metastatic melanoma. No objective antitumor responses were achieved with either RFT5-SMPT-dgA or LMB-2 in humans.[198,199]

USE OF ANTITUMOR ANTIBODIES (PASSIVE IMMUNOTHERAPY)-ANTI-GANGLIOSIDE MONOCLONAL ANTIBODIES

Unconjugated mouse monoclonal antibodies against GD2 and GD3 gangliosides induced occasional responses in Phase I studies, but frequent human antimouse antibody reactions (HAMA), along with technical difficulties in production of these antibodies, have limited their clinical development. As production of human antibodies is also cumbersome, chimeric

human-mouse antibodies were synthetized and have shown either minimal (anti-GD3 antibody KM871), or no clinical activity (murine anti-GD2 antibody ch14.18).[200,201] Although no improvement over the use of antibody alone was seen with the concurrent administration of IL-2, a conjugated form of ch14.18 with IL-2 exerted antitumor activity in a murine model, which appeared to be mediated by MHC-I-restricted $CD8^+$ cytotoxicity.[202] The combination of ch14.18 and R24 murine antibodies administered with IL-2 produced 2 PRs (in which an anti-idiotype response to ch14.18 was elicited) and 4 SD of 23 patients with melanoma.[203] Other conjugates with the toxin ricin or radioimmunoconjugate I-131 demonstrated limited clinical utility.[204] Complement dependency for antibody mediated tumor cell killing is an issue since numerous tumors express high levels of complement inhibitors such as CD59.[205]

ADJUVANT IMMUNE THERAPY

Various treatment modalities have been employed to reduce the risk of systemic recurrence in patients with intermediate (IIA), high (IIB-IIIA) and very high risk (stage IIIB-IIIC) patients. While patients presenting in stage IIA have a chance of recurrence of 20% to 30%, the rates of relapse are much higher for stages IIB, IIC, and III, averaging 40% to 80%. Early attempts using nonspecific immune adjuvants such as levamisole, Corynebacterium parvum, or BCG have not been proven in randomized trials to reduce the odds of recurrence.[206,207] The limited clinical efficacy of adjuvant vaccination has been attributed to multiple factors, including the limited immunogenicity of the epitopes used in vaccination. Immunologically mediated tumor regression may involve tumoral modulation of the antigen processing mechanism, such as the TAP-1 and TAP-2 peptide transporters, which are critical to MHC class I antigen presentation pathways.[127–129]

Interferons

Immunomodulatory effects of interferons (IFNs), along with the proven activity in metastatic disease, triggered considerable interest for testing this class of agents in the adjuvant setting. The E1684 trial randomized 287 patients between 52 weeks of high-dose interferon (HDI) versus observation. The first results of this trial reported in 1996 showed a significant improvement in RFS (1.72 y vs. 0.98, P=0.002) and OS in the HDI arm compared with observation (3.82 vs. 2.78 y P=0.02), leading to FDA approval of this treatment for stage IIB, IIC and III melanoma. A later update of data at 12.6 years of follow-up still shows a significant RFS advantage for HDI (HR=1.38, P=0.02), but a decrease in the OS benefit (HR=1.22, P=0.18), attributed partially to death from competing causes.[208] Despite considerable toxicity, use of HDI in this setting was associated with an improvement in quality of life compared to observation only. The larger E1690 trial, designed using a cure-rate model derived from the results of E1684, employed a randomized comparison of HDI and low-dose interferon (LDI) against observation alone. Again, a significant RFS benefit was recorded for HDI compared with observation (HR=1.28, P=0.025), but no impact was seen with LDI, possibly influenced by the very high salvage rate of relapsed patients in the observation group. Furthermore, neither IFN dose led to any improvement in OS. Similar to E1684, a later follow-up analysis at 6.6 years still revealed a RFS advantage (HR=1.38, P=0.02).[208] In a randomized comparison of HDI versus Gm2-klh/Qs-21 (GMK) vaccine (E1694), an initial significant advantage for both RFS and OS with HDI (HR=1.47, P=0.001 and HR=1.52, P=0.009, respectively), was maintained after 2.1 years (HR=1.33, P=0.006, and HR=1.32, P=0.04, respectively). A direct comparison of GMK alone versus GMK with either concurrent or sequential HDI (E2696) demonstrated the superiority of both HDI combinations over the vaccine alone (HR=1.75 and 1.96), reaching significance after adjusting for gender, performance status, time to resection, nodal status and age (P=0.016 and 0.03, respectively). However a survival update at 2.8 years did not confirm the advantage held initially by the two HDI groups.[208]

Composite data resulting from these four trials support the clinical benefit of HDI, with three trials demonstrating a significant improvement in disease-free survival (DFS), and 2 of them indicating an OS benefit.[208] A pooled analysis of E1684 and E1690 data at a median follow-up of 7.2 years shows a significant superiority of HDI over observation in regard to RFS (HR=1.30, $P<0.06$), but not OS (HR=1.08, $P=0.42$), which is consistent with the survival data pooled by a large meta-analysis of eight trials comprising 3,178 patients.[209] Further studies are necessary to better delineate melanoma prognostic groups and likelihood of response to adjuvant therapy before HDI can be accepted as a universal standard of care.

As discussed above in the sections regarding IL-2 and vaccine therapy, stimulation of anti-melanoma responses is associated in some cases with appearance of autoimmunity. A study by Gogas et al[210] reported a correlation between the response to HDI therapy and various manifestations of autoimmunity, including antithyroid, antinuclear, anti-DNA, and anticardiolipin autoantibodies, and vitiligo. Development of autoimmunity represented an independent prognostic factor for relapse-free and overall survival ($P<0.001$). Although lead time bias can conduct to this correlation, similar results with CTLA-4 antibodies indicate an association between autoimmune phenomena (thyroiditis, hypophysitis, enteritis, hepatitis, and dermatitis) and prolonged survival in metastatic disease.[146,155]

Alternative interferon schedules have been designed in order to avoid toxicities associated with high-doses or prolonged courses of therapy. No survival benefit was achieved using an induction phase of 10 MU s.c. daily 5 days per week followed by 2 years of LDI.[211] Likewise, two studies employing two and three years of LDI (3 MU s.c. three times a week), had no DFS or OS benefit compared to observation.[212,213] Reduction of the duration of adjuvant HDI to 3 months (20 MU/m^2 trice weekly) did not produce a median OS benefit (6.6 y for IFN-α 2a and 5.0 y for observation, $P=0.40$), but a possible improvement was suggested for selected high-risk node-positive patients (OS 4.1 vs. 2.7 y $P=0.44$). A comparison between one month induction therapy versus one full year of high-dose adjuvant interferon was reported in 364 patients with stage IIB, IIC, and III melanoma treated no later than 56 days of curative surgery with no significant differences in OS and RFS between the regimens of 1 month and 1 year of treatment.[214]

Based on data showing synergistic antitumor efficacy in vitro and in vivo,[215] a combination of LDI-α2b and low-dose IL-2 was tested in 225 node-negative, pT3 and T4 patients, and demonstrated no superiority in regard to DFS or OS compared to LDI-α2b alone.[216] Adjuvant treatment for high-risk stages IIA to IIIB with DTIC and low-dose natural interferon-a resulted in a significantly higher 7-year calculated OS rate of 51 versus 30% ($P=0.007$), with a greater benefit on late mortality, especially in high-risk patients.[217] However, this data needs to be further confirmed.

Pegylated IFN-alpha-2b (PEG-IFN-alpha-2b) has raised interest for the chronic adjuvant treatment of melanoma given its convenient administration and positive outcomes recorded in the European trials. In a study of 1256 patients with stage III melanoma, PEG-IFN-alpha-2b (n=627) induction with 6 micrograms/kg/wk for 8 weeks followed by maintenance with 3 micrograms/kg/wk was administered against placebo for an intended total duration of 5 years. After 3.8 years of follow-up, the risk for recurrence-free survival (RFS) was reduced by 18% (hazard rate=0.82; $P=0.01$), along with an expected negative effect on global quality of life score.[218] The exposure to Peg-IFN alpha-2b appears to be sustained during the long-term adjuvant treatment in melanoma, which is consistent with the European Organization for Research and Treatment of Cancer 18991 data indicating a significant, sustained, relapse-free survival benefit.[219]

High-dose Interferon (HDI) administered neoadjuvantly in doses of 10 MU/m^2 three times weekly resulted in clinical responses in 55% of patients, and 50% of patients had no recurrence of their disease at over 1.5 years of follow-up. In this patient population of stage IIIB melanoma, clinical responds were associated with larger amounts of tumor infiltrating $CD3^+$ and $CD11c^+$ lymphocytes.[220] Neoadjuvant biochemotherapy with cisplatin, vinblastine, dacarbazine, interleukin-2 and interferon-alpha 2a produced a 50% response rate and a 65% DFS at 31 months,[221] and a phase III intergroup trial comparing a short intensive course of biochemotherapy (IL-2, IFN-α, cisplatin, DTIC and vinblastine) with standard HDI is currently addressing the high-risk stage III patients. Equivalent clinical results with less toxicity than HDI were obtained with a combination of intermediate-dose IFN and Melacine vaccine in stage III melanoma (RFS 31 vs. 25 mo, P=0.85).[222]

Microarray analysis of PBMCs gene induction in vitro may be a useful predictor of the in vivo response of T cells, NK cells or monocytes to treatment with IFN-α. Various genes involved in immune responses, nucleic acid binding and metabolism, protein catabolism, or cyclin-dependent protein kinase activity (OAS2, OASL, HERC5, ISG20, IFI44, LIR7, LGP2, MT1H, MT2A, N4BP1, PLSCR1, USP18, TREX1, ZCCHC2) are activated in both settings, therefore being potential markers of patient response to interferon.[223]

Vaccines

Several melanoma-specific vaccines have been prepared from whole cells,[224–226] or from antigens shed from allogeneic cell lines.[227] A prolongation of survival in comparison to historical controls or to patients not developing an immune response to the vaccine was reported in the initial studies. However, randomized phase III studies did not confirm a significant benefit.

In a 689 patients trial comparing an allogeneic melanoma cell lysate vaccine, Melacine, to observation in patients with stage II disease, a similar 5-year disease-free survival was reported (65% for the vaccine and 63% for observation arm).[228] An OS analysis could only be performed in a follow-up study, which showed a survival advantage for the HLA-$A2^+$ and/or $C3^+$ group, but no significant benefit for the overall cohort of patients.[229]

Canvaxin is an allogeneic whole cell vaccine that was tested in double blind trials of post resection patients with high probability of relapse. Initial nonrandomized studies demonstrated promising results.[230] 2602 AJCC stage III melanoma patients who underwent lymphadenectomy were enrolled in a study where 935 received Canvaxin vaccine between 1984 and 1998, while 1667 historical controls did not. A significantly higher median OS and 5-year OS was observed in patients receiving the vaccine (56.4 vs. 31.9 mo and 49% vs. 37%, respectively; P=0.0001). The difference was maintained when patients treated with Canvaxin were matched with non-PV patients by six covariates forming 739 pairs.[231] Another study involving 150 stage IV melanoma patients treated adjuvantly with Canvaxin after surgical resection demonstrated induction of delayed-type hypersensitivity (DTH), which correlated with the OS (39% for vaccinated and 19% for non-vaccinated patients) (P=0.0001). In a multivariate analysis model, vaccine therapy was the most significant prognostic variable (P=0.0001).[232] However, subsequent prospective randomized trials showed less favorable results, with a trend towards decreased survival in treated patients. Among 1656 patients with resected stage III and IV melanoma, Canvaxin failed to demonstrate improvements in overall survival (OS), as compared to BCG.[233]

Similarly, a polyvalent, shed-antigen Bystryn vaccine did not produce a significant statistical improvement in median OS, even after adjusting for risk factors. Thirty-eight patients with stage III melanoma with a particularly poor prognosis were immunized intradermally in a 2:1 vaccine/placebo ratio every 3 weeks×4, monthly×3, every 3 months×2, and then every 6

months for 5 years or until disease progression. At 2.5 years median length of observation, the median time to disease progression was 2.5 times longer in the active arm (*P*=0.03). Median overall survival was 40% longer in the active treatment group (3.8 vs. 2.7 y, *P*=nonsignificant).[234]

Interest in targeting more specific surface antigens has resulted in vaccines derived from GM2 gangliosides, a well-defined melanoma associated antigen. Anti-GM2 antibodies have been detected in approximately 5% of melanoma patients, and their presence was associated with an increase in the relapse-free survival (RFS).[235] An IgM antibody response was obtained in 85% of patients with resected stage III melanoma who were immunized with purified GM2 adherent to BCG. An improved DFS was observed for patients with anti-GM-2 titers >1:40, but such a benefit could not be confirmed in a subsequent randomized phase III trial comparing GM2+BCG vaccine with BCG alone.[236] However, when the six patients who produced GM2 antibodies before randomization were excluded, an increased DFS of 23% (*P*=0.02) and a trend toward longer OS were observed for the GM2+BCG. Another phase III randomized trial comparing the efficacy of high-dose interferon alfa-2b therapy (HDI) versus vaccination with GM2 (GMK) demonstrated an overall benefit for HDI in terms of RFS and OS in melanoma patients. Antibody responses to GM2, however, were associated with a trend toward improved RFS and OS.[237]

However, no survival benefit was noted in two large phase III trials which used adjuvant GM2-KLH with QS21 adjuvant. A negative effect on survival might have been observed in the E1694 trial, where 880 patients with resected stage IIB and III melanoma have been randomized between GM2-KLH vaccine and HD IFNa-2b. Lower RFS (relapse-free survival) and OS (overall survival) were seen in the GM2-KLH arm.[237] A negative effect on the rate of development of distant metastases and a lower OS (HR 1.57, *P*=0.03) were observed in an European study European Organization for Research and Treatment of Cancer 18961 involving 1314 patients with resected stage II (T3-4N0M0) disease who were randomized between the GM2-KLH vaccine and placebo, although the disease-free survival was similar in the two groups.[238] Both studies were closed prematurely because of the apparent detrimental effect on survival of the GM2-KLH vaccine.

The most immunogenic peptides among 12 cancertestis and melanocyte differentiation proteins tested are tyrosinase, gp100, MAGE-A1, and MAGE-A10, and it was determined that administration of multiple peptides is safe and immunogenic.[239] An ongoing Intergroup trial (E4697) is currently testing vaccination with HLA-A2-restricted peptides (tyrosinase, gp210M, and MART-1), with GM-CSF used either alone (in HLA-A2- patients), or added to the peptide vaccine for potential synergy.

Anti-ganglioside Monoclonal Antibodies

A humoral response can be generated against the monoclonal antibody active sites. Anti-GD3 antibodies were induced in 3 of 14 patients immunized with BEC2, an anti-idiotypic monoclonal antibody that mimics GD3 and BCG, but the immunogenicity of anti-idiotypic monoclonal antibodies has generally remained low. A 71% survival and a 64% disease-free survival at 2.4 years of follow-up were considered to be encouraging results.[240]

TLR Agonists

In a study involving 24 patients with stage I to III melanoma, local administration of PF-3512676 (formerly CpG 7909), a synthetic deoxycytidyl-deoxyguanosine oligonucleotide which activates TLR-9, has induced melanoma-specific $CD8^+$ T-cell responses against at least one melanoma-associated antigen in the draining lymph node in 50% of the patients receiving the compound, vs. none of the control patients injected with

saline (P=0.01). Clinical response has been associated with enhanced local and systemic melanoma-specific $CD8^+$ T-cell reactivity and NK cell mobilization.[241] Intradermally injected PF-3512676 appears promising as an adjuvant therapy for early-stage melanoma, being associated with increased dendritic cell activation, enhanced type I cytokine secretion, and a significant reduction in $CD4^+CD25^+$ Tregs in sentinal lymph nodes.[242]

Granulocyte-macrophage Colony Stimulating Factor (GM-CSF)

The use of GM-CSF in the adjuvant treatment of melanoma is based on its differentiating activity on DC and formation of cytotoxic macrophages. A better survival rate of 38.0 versus 12.2 mo was obtained in a single-arm study of s.c. GM-CSF (P<0.001) compared to historical controls, with only one of 48 node-positive patients discontinuing the drug due to a grade 2 injection site reaction.[243] The increase of IL-2 receptor expression on T-lymphocytes in response to GM-CSF was postulated to act synergistically with formation of LAK and TIL cells by IL-2. Early results in a trial assessing the efficacy of this combination indicated a DFS of 93.7% at a median follow-up of 14 months, and a good tolerability of the combination.[244] GM-CSF has been reported to also possess immune suppressive effects depending on concentration, therefore, a word of caution must be added when discussing these trials.[84]

CONCLUSIONS

Progress in identifying tumor epitopes of heightened immunogenicity and advances in deciphering the homeostasis of immune responses has lead to a new age of melanoma immunotherapy. Recent important steps represent the recognition of tumor immune evasion mechanisms, which resulted in the clinical use of anti-CTLA4 and anti-PD1 antibodies; understanding of the importance of costimulatory signals, which was translated into the use of CD40 agonist mAbs; and appreciation of the importance of innate immune activation, causing investigators to seek stimulation of dendritic cells by various TLR agonists. Defining of the role played by immune adjuvants and the influence of booster doses has represented important additions to the development of anti-tumor immunology. Finally, the recognition of the role played by Treg cells in the formation of immune responses and their interference with immune effectors resulted in new strategies to deplete or interfere with their function.

Through accepting that immune-based approaches lead to limited responses, which often do not exceed a 15% response rate threshold, the hypothesis of a decisive role played by tumor-induced immunosuppression, has been gaining acceptance. Such understanding explains the poor clinical results observed even in well-designed, controlled clinical studies. However, much disappointment resulted over time as the results of a plethora of small phase I and II trials designed to assess the safety and biologic properties of immunotherapeutic agents appeared to have created breakthroughs in clinical responses. A delay in implementing the latest immunological knowledge in Phase III trials, as well as the absence of adequate control study populations further flawed the scientific and clinical value of many of the recent trials. In addition to these obstacles comes the fact that the much-sought correlation between tumor responses with the presence of immunological responses is also not reliably demonstrated across studies. However, this yet faint association raises hope that the clinical efficacy of immunotherapy may be increased once the mechanisms of tumor suppression are better understood and addressed. This also highlights the urgent need to validate standardized biomarker/immune monitoring methods so that trials can be accurately compared, as well as to develop more accurate diagnostic biomarkers. Currently the International Society for the Biological Therapy of Cancer (iSBTc) has initiated in collaboration with the United States Food and Drug Administration (FDA) to address these two issues through a systematic analysis of available technologies. Two working groups

have been created[245] with the goal to perform high-throughput screening of clinical samples in order to identify predictors of immune responsiveness, clinical responsiveness, and survival in an era where the response evaluation criteria in solid tumors criteria may not be entirely reflective of the clinical benefit, identifying markers that predict the risks of toxicity to treatment, and identifying mechanistic biomarkers which will help characterizing the mechanism of action of immunotherapeutic approaches. A strategy to observe the common modifications that occur during response to therapy was proposed, which consists of analyzing samples relevant to the genetic background of the patients, the modified phenotypes of immune cells in relation to the natural evolution of the neoplasia, and the tumor response at local and distant sites. Identified promising immune monitoring techniques for the future are PET scans of activated T cells, or analysis of the proteins produced during immune activation and tumor response, which can be performed either non-invasively or through a minimal peripheral blood collection.[245]

The possibility to engineer better immune interactions and to boost positive feedback loops predicts a new coming of age of immunotherapy. Demonstration of immune responses to tumor-associated antigens, along with the possibility to follow the T-lymphocyte activation during immune stimulation in fact opens a new age in testing and enhancing immune stimulating approaches with proficiency. The contribution of fundamental research, along with the discovery of more potent immune stimulation strategies, will probably be able to separate the anti-tumor responses from the generation of autoimmunity. Insidiously coming of age, melanoma immunotherapy has a future which is ultimately dependent on the understanding of the contradictory and complex influences that govern the immune responses, and in particular the immunosuppressive barriers.

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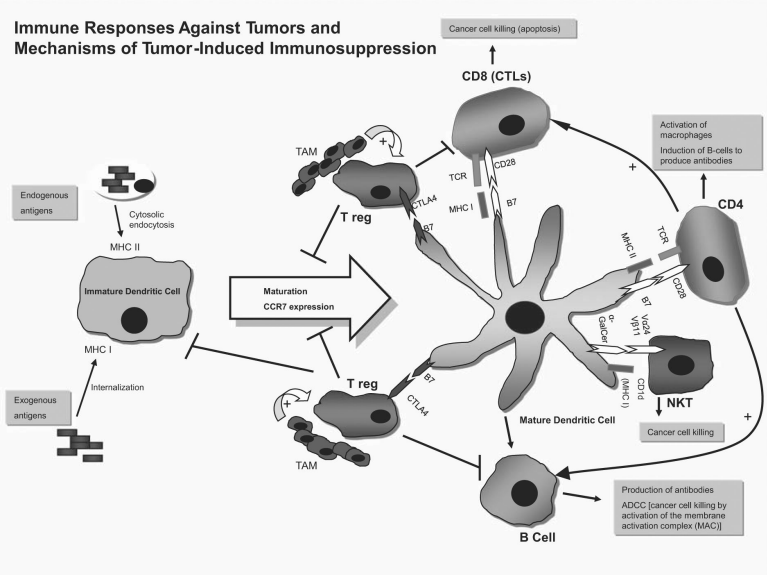

FIGURE 1.

Role of Dendritic Cells (DCs) and Mechanisms of Tumor-Mediated Immunosuppression (schematic). The activation of immature dendritic cells (iDCs) is followed by migration to lymphatic nodes, sites of transformation to mature dendritic cells. The uptake and processing of antigens by DCs is the first step in the activation of T-effectors, NK-cells, $CD4^+$-cells, and B-cells. Two different pathways involve the processing of endogenous (intracellular) and exogenous antigens (apoptotic cancer cells). Activation of cytotoxic T cells involve antigen cross-presentation of tumor antigens by DCs. DCs migration to lymph nodes (LNs) occurs through a CCR7 gradient. Subsequently, DCs complete the maturation process by expressing surface MHC-antigen complexes. Mature DCs are able to induce various cellular effectors against cancer cells, such as $CD4^+$ (MHC II-restricted) and cytotoxic $CD8^+$-cells (MHC I-restricted). Activation of NKT cells is made through a MHC-I restricted interaction between the Va24 T-cell receptor chain with the glycolipid α-GalCer, resulting in tumor cell killing. Other consequences of NKT-cell activation consist of $CD8^+$-cell and NK cell stimulation, with consequent anti-tumor effects. Immune activation is however interfered by the inducement of immune tolerance by the tumor, or by the immature dendritic cells (DC) which lack proper antigenic stimulation. T-regulatory cells can interfere with DC-effector T-cell interactions and inhibit the DC activation, with resultant immune anergy. Tregs are activated by tumor-associated macrophages (TAMs) and interfere with the process of DC polarization. One immunosuppressive effect exerted by the T regs is mediated through CTLA4 receptor interaction with the B7 (CD80/CD86) ligands on the surface of T cells and DCs. Several other molecules (suppression of cytokine-signaling1, PD1) mediate tumor-induced immunosuppresson. TAM can produce an incomplete polarization of iDCs through various soluble facrors.

TABLE 1

Clinical Use of Cytokines in Melanoma

Cytokine Regimen	Stage	Clinical Response	Common AE	References
IL-2 2 cycles at 100,000 U/kg IV q4 h×5d	IV	OR 10/47 (CR 2/47, PR 8/47)	MI (6%)	Parkinson et al[5]
IL-2 continuous infusion at 12×10^6 U/m^2 over 24 h×4d/wk for 4wk q6 wk)	IV	OR 7/31 (CR 1/31, PR 6/31)	Reversible hepatic or renal insufficiency (16%)	Legha et al[6]
IL-2±IFN-ά IL-2 monotherapy: dosed at 6×10^6 U/m^2 q8 h, maximum 14 doses IL-2+IFN-ά: dosed at 4.5×10^6 U/m^2+IFN-α 3×10^6 U/m^2	III–IV	IL-2 monotherapy: OR 2/44 (CR 0/44, PR 2/44) IL-2+IFN-α OR 4/41 (CR 0/41, PR 4/41)	3 treatment-related deaths; trial terminated early based on predefined early stopping rules	Sparano et al[7]
IL-2±vaccine dosed at 720,000 IU/kg IV q8 h, maximum 10–15 doses	IV	IL-2 monotherapy: OR 39/305 (CR 13/305, PR 26/305) IL-2+vaccine: OR 59/379 (CR 13/379, PR 46/379)	Chills, rigors, malaise, nausea, vomiting, diarrhea	Smith et al[8]
IL-2+TMZ dosed at 600,000 U/kg IV, maximum 14 doses over 5 d (after TMZ 75 mg/m^2 PO q day×3wk)	IV	OR 5/31 (CR 2/31, PR 3/31), SD 16/31	Hyperbilirubinemia (24%), diarrhea (5%), oliguria (3%), leucopenia (13.2%), thrombocytopenia (7.9%)	Agarwala et al[9]
IL-2+gp100 vaccine dosed at 600,000 U/kg q8 h, max doses over 5 d	IV	OR 20/121 (CR 11/121, PR 9/121)	Local swelling and discomfort	Sosman et al[10]
IFN-α+TMZ versus TMZ alone IFN: 5 MU/m μg SC thrice weekly TMZ: 200 mg/m^2 PO days 1–5, q28 d	IV	TMZ monotherapy OR 18/134 (CR 3/134, PR 15/134) IFN-α+TMZ OR 33/137 (CR 11/137, PR 22/137)	Leukopenia (56%), thrombocytopenia (47%), anemia (40%)	Kaufmann et al[11]
INF-α2b+IL-2+CDDP+DTIC	IV	OR 25/57 (CR 14/57, PR 11/57)	Cytopenias	Bar et al[12]
PEG-IFN-αa2a+DTIC IFN: 180 μg SC qweek up to 25 wk DTIC: 50 mg/m^2 IV q3 wk up to 25 wk	IV	OR 6/25 (CR 2/25, PR 4/25)	Leukopenia (35%), nausea (32%), headache (18%), diarrhea (14%)	Hauschild et al[13]
PEG-IFN-α-2b+TMZ IFN: 100 μg SC qweek TMZ: 200mg/m^2 PO days 1–5, q28 d	IV	OR 21/116 (CR 2/116, PR 19/116)	Thrombocytopenia (20.7%), leukopenia (23.3%)	Spieth et al[14]
IL-12+IL-2 rhIL-12: 100 to 500ng/kg biweekly×4wk cycles IL-2: 1 to 3 MU/m^2 SC thrice weekly×4wk cycles	IV	OR 1/7 (CR 0/7, PR 1/7)	Leukopenia, neutropenia, hepatoxicity	Alatrash et al[15]
Onco $VEX^{GM\text{-}CSF}$ monotherapy	II, IV	OR 6/31 (CR3/31, PR 3/31), SD4/31	Mild flu-like symptoms	Senzer et al[16]

AE indicates adverse events; CDDP, cisplatinum; CR, complete response; DTIC, dacarbazine; IFN, interferons; IL, interleukin; IV, intravenous; MI, myocardial infarction; $OncoVEX^{GM\text{-}CSF}$, second-generation oncolytic Herpes Simplex virus (HSV) expressing granulocyte macrophage colony-stimulating factor; OR, overall response; PEG, pegylated; PO, orally; PR, partial response; rhIL, recombinant human; SC, subcutaneous; TZM, temozolomide.

TABLE 2

Clinical Trials Assessing Dendritic Cell-based Vaccines for Patients With Melanoma

Regimen	Stage	Clinical Response	Common AE	References
Peptide, autologous tumor lysate	III–IV	OR 5/16 (CR 2/16, PR 3/16), SD 0/16	Well tolerated	Nestle et al[96]
Peptide	IV	OR 7/17 (CR 3/17, PR 4/17), SD 3/17	Progressive vitiligo (12%)	Banchereau et al[97]
Peptide	IV	OR 1/16 (CR 0/16, PR 0/16), SD 2/16	Arthralgia (33%), diarrhea (13%)	Lau et al[98]
Peptide	IV	OR 1/16 (CR 1/16, PR 0/16), SD 8/16	Injection site reaction, transient fever	Schuler-Thumer et al[99]
Peptide	IV	OR 3/19 (CR 1/19, PR 2/19), SD 1/19	Mild flu-like symptoms (21%), vitiligo (16%)	Smithers et al[100]
Peptide versus peptide+ GM-CSF	III–IV	OR 1/13 (CR 0/13, PR 1/13), SD 1/13 OR 2/13 (CR 0/13, PR 2/13), SD 2/13	Transient injection site reactions, diarrhea, pruritis	Slingluff et al[101]
Autologous tumor lysate	IV	OR 6/17 (CR 3/17, PR 3/17), SD 0/17	Transient flu-like symptoms	O'Rourke et al[102]
Peptide	II–IV	OR 1/9 (CR 1/9, PR 0/9), SD 1/9	Progressive vitiligo (11%)	Ribas et al[103]
Allogeneic tumor lysate	IV	OR 0/60, SD 4/60	Transient injection site reactions	Gorin et al[104]
Peptide	IV	OR 2/6 (CR 1/6, PR 1/6), SD 1/6	Transient hepatic dysfunction (33%)	Akiyama et al[105]
Allogeneic Colo829 melanoma cell line	IV	OR 2/20 (CR 1/20, PR 1/20), SD 2/20	Vitiligo (10%)	Palucka et al[106]
Autologous tumor lysate, autologous tumor homogenate	III–IV	OR 2/21 (CR 1/21,PR 1/21), SD 6/21	Transient flu-like symptoms, injection site reactions, vitiligo (9%)	Ridolfi et al[107]
Allogeneic tumor lysate	III–IV	OR 1/9 (CR 1/9, PR 0/9), SD 1/9	Injection site reaction (33%), anorexia (20%), asthenia (20%)	Salcedo et al[108]
Allogeneic tumor lysate (M44, SK-MEL 28, COLO 829)	IV	OR 2/33 (CR1/33, PR 1/33)	Age-related macular degeneration in one patient	Ross et al[109]
Autologous tumor lysate	IV	OR 6/33 (CR 3/33, PR 3/33), SD 0/33	Well tolerated	O'Rourke et al[110]
Autologous tumor lysate	III–IV	OR 2/42 (CR 1/42, PR 1/42)	Flu-like symptoms, transient injection site hyperemia and pruritis	Petenko et al[111]
Dendritic/tumor cell hybrid (dendritoma) + IL2	IV	NED 3/15, SD4/15	Well tolerated	Wei et al[112]

CR indicates complete response; GM-CSF, granulocyte-macrophage colony stimulating factor; OR, overall response; PR, partial response.

TABLE 3

Clinical Trials of CTLA-4 Antibody Blockage to Stimulate Immune Responses in Melanoma

CTLA-4 Blockade	Stage	Clinical Response	Common AE	References
Ipilimumab + gp100 peptide vaccine	IV	OR 3/14 (CR 2/14, PR 1/14)	Dermatitis (21%), vitiligo (14%), hypophysitis (7%), enterocolitis (7%)	Phan et al[146]
Ipilimumab ± DTIC	IV	Monotherapy: OR 2/37 (CR 0/37, PR 2/37), SD 4/37 + DTIC: OR 6/35 (CR 2/35, PR 2/35), SD 4/35	NA	Fischkoff et al[147]
Ipilimumab + IL2	IV	OR 8/36 (CR 3/36, PR 5/36)	Enterocolitis (11%), arthritis (3%), uveitis (3%)	Maker et al[148]
Ipilimumab + gp100 vaccine	IV	OR 7/56 (CR 2/56, PR 5/56)	Colitis (12%), dermatitis (7%), enterocolitis (2%)	Attia et al[149]
Ipilimumab monotherapy	III, IV	OR 4/88 (CR 1/88, PR 3/88), SD 8/88	Rash, pruritis, diarrhea, colitis	Weber et al[150]
Ipilimumab (formerly MDX - 010) ± gp100 peptide vaccine	IV	OR 23/139 (CR 3/139, PR 20 /139)	Dermatitis (30%), enterocolitis (3%), hypophysitis (2%)	Downey et al[145]
Tremelimumab (CP-675, 206) monotherapy	III–IV	OR 4/34 (CR 2/34, PR 2/34), SD 4/34	Diarrhea, dermatitis, vitiligo (2%)	Ribas et al[151]
Tremelimumab: 10 mg/kg q1mo versus 15 mg/kg q3mo	III–IV	10 mg/kg q1 mo: OR 2/44 (CR 1/44, PR 1/44) 15mg/kg q3 mo: OR 3/45 (CR 1/45, PR 2/45)	Diarrhea (5%), colitis (2%)	Ribas et al[152]
Tremelimumab monotherapy	III–IV	OR 20/241 (CR 0/241, PR 20/241)	Diarrhea (11.4%), fatigue (2.4%), colitis (2.0%)	Kirkwood et al[153]

CR indicates complete response; CTL, cytotoxic T-lymphocyte; DTIC, dacarbazine; IL, interleukin; NA, not available; OR, overall response; PR, partial response.

TABLE 4

Clinical Trials Using TLR-9 Agonist Stimulation of Immune Responses in Melanoma

TLR Agonists	Stage	Clinical Response	Common AE	References
PF-3512676+MAGE-3 vaccine	IV	OR 1/12 (CR 0/12, PR 1/12), SD 2/12	Injection site reactions, fever, fatigue	Kruit et al[167]
PF-3512676 (subcutaneous)	IV	OR 2/20 (CR 0/20, PR 2/20), SD 3/20	Injection site reactions, fever, arthralgia	Wagner et al[168]
PF-3512676 (subcutaneous) ± DTIC	IV	OR 7/93 (CR 0/93, PR 7/93)	Injection site reactions, fever, athralgia	Wagner et al[169]
PF-3512676 (subcutaneous)	IV	OR 2/20 (CR 0/20, PR 2/20), SD 3/20	Injection site reactions (40%), mild flu-like symptoms (10%)	Pashenkov et al[170]
PF-3512676 (intralesional)	IV	OR 1/5 (CR 1/5, PR 0/5)	Well tolerated	Hofmann et al[171]

CR indicates complete response; DTIC, dacarbazine; OR, overall response; PR, partial response; TLR, Toll-like receptor.