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REVIEW ARTICLE

Metastatic Melanoma – A Review of Current and Future Treatment Options

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Despite advances in treatment and surveillance, melanoma continues to claim approximately 9,000 lives in the US annually (SEER 2013). The National Comprehensive Cancer Network currently recommends ipilumumab, vemurafenib, dabrafenib, and high-dose IL-2 as first line agents for Stage IV melanoma. Little data exists to guide management of cutaneous and subcutaneous metastases despite the fact that they are relatively common. Existing options include intralesional Bacillus Calmette–Guérin, isolated limb perfusion/infusion, interferon-α, topical imiquimod, cryotherapy, radiation therapy, interferon therapy, and intratumoral interleukin-2 injections. Newly emerging treatments include the anti-programmed cell death 1 receptor agents (nivolumab and pembrolizumab), anti-programmed death-ligand 1 agents, and oncolytic vaccines (talimogene laherparepevec). Available treatments for select sites include adoptive T-cell therapies and dendritic cell vaccines. In addition to reviewing the above agents and their mechanisms of action, this review will also focus on combination therapy as these strategies have shown promising results in clinical trials for metastatic melanoma treatment. ***Key words: Stage III; Stage IV; IL-2; CTLA-4; PD-1.***

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There have been over 1,500 academic papers published on the use of traditional chemotherapy for metastatic melanoma with no evidence for survival benefit. Recent advances in strategies to either block signal transduction (BRAF inhibition) and/or enhance anti-tumor immune responses (CTLA-4 blockade) have led to the first clinical trials in history where treatment has been associated with a survival benefit. To date, these survival benefits have only been modest but they do pose a proof of concept: highly targeted therapeutic strategies for cancer can increase survival (1).

Patients with Stage IV melanoma continue to have a poor prognosis, with a mean survival of 8–10 months in large cohort analysis studies (2). For a more accurate prognosis, patients with stage IV disease can be further subdivided into those with only cutaneous metastases (IVa), lung metastases (IVb), or other visceral metastases (IVc) to yield associated 5-year survival rates of 18.8%, 6.7%, and 9.5%, respectively (2). Patients with cutaneous melanoma metastases (Stage IIIb or IVa) are frequently treated with local excision; however this treatment approach does not address the microscopic in-transit malignant cells present which often give rise to future tumors. BRAF inhibition or cytotoxic T-lymphocyte antigen 4 (CTLA-4) blockade have emerged as useful options for IVb and IVc patients, but it is unclear if these agents should be applied to patients with stage IIIb or IVa disease. Herein, we will review all treatment modalities and explore future directions for patients with advanced melanoma including patients with cutaneous only metastases.

SURGERY

Cutaneous melanoma metastases come in 3 varieties: satellite within 2 cm from the primary tumor, in transit which are > 2 cm from the primary tumor but within the same region as the primary, and distant metastases outside of the region of the primary tumor. Both satellite and in transit metastases occur via dermal lymphatic circulation and are designated as N2c in the TNM staging system (2). Patients with N2c disease without nodal metastasis have a 60% 5-year survival rate versus 36% for those with synchronous nodal metastases (3). Distant dermal metastases imply a hematogenous route of travel, allowing for cutaneous deposition of melanoma cells far from the primary site. Currently there is no gold standard therapy for dermal metastases but surgery is an attractive option because patients are quickly rendered "disease-free" with relatively limited associated morbidity. In contrast, systemically administered therapies require prolonged treatment courses to achieve relatively inferior local response rates. In addition to effective palliative

management of local disease, excision of systemic, particularly solitary lung melanoma metastases may also increase patient survival (4). Data in support of this is mainly from patients with advanced melanoma. Wong et al. (5) reported a 5-year survival rate of 20% in 144 patients who underwent surgical resection of non-regional melanoma metastases and a phase II trial by the Southwest Oncology Group reported overall 3- and 4-year survival rates of 36% and 31%, respectively, in stage IV melanoma patients (6). Other retrospective studies have demonstrated similar numbers. However, selection bias could have contributed to the excellent survival rates reported in these studies.

Because surgery alone cannot identify and address microscopic metastases, clinical trials are testing combination strategies such as surgical resection in conjunction with systemic targeted therapies. For example, prior to the advent of targeted immunotherapy, a Malignant Melanoma Active Immunotherapy Trial (MMAIT) reported 5-year overall survival rates of 42.3% and 63.4% (stage IV and III, respectively) using postoperative adjuvant immunotherapy with Bacillus Calmette-Guerin (BCG) and an allogeneic melanoma vaccine (MCV) (7). More recent approaches are outlined in later sections.

ISOLATED LIMB PERFUSION/ISOLATED LIMB INFUSION

Traditionally, locally recurrent metastatic melanoma of the limb has been treated with surgery, chemotherapy, and finally amputation as the cancer progresses. More recently, isolated limb perfusion (ILP) has emerged as an effective limb salvaging therapy for widespread regional cutaneous and subcutaneous metastases. In ILP, high local doses of chemotherapy are delivered to the patient under an extracorporeal circuit that isolates the affected limb, thereby avoiding systemic toxicity. The rationale of this delivery system is that high local doses of cytotoxic agents can be delivered with minimal systemic adverse events. Early trials have reported overall response rates of 30–60% with half achieving complete remission (8).

ILP is most commonly performed with the alkylating agent melphalan, ideally under hyperthermic conditions. Hyperthermia enhances the cytoxicity of melphalan and increases its uptake into neoplastic cells (9–11). Addition of hyperthermia to melphalan perfusion has been found to increase overall response rates to 80–90% and complete response rates to 25–60%.

More recently, the addition of tumor necrosis factor (TNF) and interferon (IFN)-γ to melphalen perfusion has been found to increase overall response to ILP. In a recent phase III trial, the combination of TNF, IFN-γ, and melphalan achieved a complete response rate of 80% (12). Subsequent studies that included TNF in combination with melphalan all yielded very good response rates, with the exception of one randomized control trial (13) which has received criticism for the timing of its endpoint analysis and uniformly low response rates in both groups. In a systematic review of 22 studies, 556 ILP's with TNF + melphalan yielded a median complete response of 68.9% compared to 46.5% with 562 ILP's with melphalan alone (14).

Although ILP achieves high initial response rates, a lack of comparative trials precludes any reasonable attempt at characterizing survival benefit. Many believe that ILP does not impart an overall survival or disease-free survival benefit (15). One center's experience with 103 patients who had received ILP reported a 5-year overall survival rate of 26% and disease-free survival of 12% (16).

A similar technique, isolated limb infusion (ILI), was developed in the 1990's as a less invasive alternative to ILP. ILI differs from ILP in that it is performed percutaneously rather than surgically (17). Although no trial has compared the two, ILI has produced similar response rates to ILP and appears to be a viable alternative in patients who are unfit for or who do not wish to undergo ILP.

CRYOTHERAPY

Cryotherapy with liquid nitrogen was previously used as a non-invasive targeted treatment for limited cutaneous metastatic melanoma (18). Theoretically, this therapy results in tumor antigen release through local trauma to the area and thus has the potential to elicit a systemic anti-melanoma immune response. However, the tumor-specific immune response following cryotherapy seems to be inferior when compared to other destructive modalities (19).

RADIATION THERAPY

Radiation therapy (RT) is used in 1–6% of patients with melanoma in the US. It is commonly used as adjunct therapy or as palliative therapy, particularly for patients with brain metastases, delivered as sterotactic gamma knife radiosurgery (20). In the setting of inoperable disease, RT is a reasonable option for palliation. In one study employing high dose therapy, reported response rates ranged from 67–100%, although in practice this is seldom observed (21).

One potential benefit of RT over surgical management is that RT can possibly induce an abscopal (away from the target) effect in which both the treated tumor, as well as non-irradiated sites, show a response to the treatment. Although the mechanism for the abscopal effect is not well characterized, it is thought to be immune-mediated. RT can promote cross-priming, the process by which

released tumor antigens are presented in the context of MHC class I molecules by dendritic cells. Locally activated $CD8^+$ T cells can then migrate to tumors at distant sites and induce tumor lysis (22). Recent phase I trials of stereotactic body radiotherapy in combination with IL-2 in 7 patients with metastatic melanoma resulted in a 71.4% response rate (23). Currently, patients are being recruited for a clinical trial to compare IL-2 alone versus IL-2 with RT (NCT01416831).

BRAF INHIBITION

The *BRAF* gene encodes for a serine/threonine kinase that participates in the MAP kinase pathway (24). Mutations in the *BRAF* gene are present in 40–70% of melanomas, leading to constitutive activation and uncontrolled cellular proliferation. Vemurafinib (Table I) and dabrafenib are BRAF inhibitors specific to melanomas harboring the BRAF V600E and V600E/K mutations, respectively (25–27). A phase III trial compared vemurafinib to dacarbazine in patients with previously untreated, unresectable stage III/IV melanoma (28). The vemurafenib arm demonstrated superior overall survival (86% versus 64% at 6 months) and progression-free survival (median 5.3 months versus 1.6 months when compared to dacarbazine alone). The overall response rate was 48% for vemurafenib and 5% for dacarbazine. Given vemurafenib's superior response, the trial was stopped early at interim analysis and crossover from dacarbazine to vemurafenib was suggested (28). A subsequent single-arm phase II trial with a median follow-up of 12.9 months demonstrated a median overall survival of 15.9 months for vemurafenib-treated melanoma patients; 32.9% of the vemurafenib responders (complete and partial) maintained their response through the end of the trial (1). A combination of surgical excision with adjuvant vemurafenib is currently being studied in an ongoing trial (NCT01667419), and neoadjuvant therapy to decrease tumor volume prior to surgical intervention in otherwise inoperable tumors is also an area of active investigation.

Table I. *Therapies used to treat melanoma*

Treatments	Evidence[a]	Reference
BRAF inhibitor		
Vemurafenib	1	1, 25, 28
Intralesional IL-2	4, 5	37–44
Adoptive cell therapy	6	100–103
Anti-CTLA4 therapy		
Tremlimumab (CP-675,206)	1	104, 105, 111
Ipilimumab (MDX-010)	1	107, 110, 112, 114
Ipilimumab and Dacarbazine	1	113
Anti-PD-1		
Pembrolizumab	1	117, 118
Nivolumab	4	116

[a]Evidence: 1: Randomized controlled trial; 2: Case series; 3: Case report; 4: Phase I study; 5: Phase II study; 6: Cohort study.

Although the increase in survival with vemurafenib alone has been modest, combination therapies using this agent along with other targeted therapies and immunotherapies, are currently under investigation. One approach using targeted therapies will require understanding the mechanisms involved in the development of vemurafenib resistance and subsequently design multidrug regimens that block these survival pathways. In fact, identifying these resistance pathways in a given patient may allow for a more "personalized medicine" approach (29). A phase II clinical trial of BRAF V600E-positive patients treated with oral vemurafenib found that the reactivation of MAPK, as observed by elevated ERK1/2 phosphorylation levels, was due to the appearance of secondary mutations in NRAS and MEK, such as NRASQ61, MEK1Q56P, and MEK1E203K (30, 31); however a preexisting MEK1 mutation prior to the use of vemurafenib did not predispose to resistance (32). Additional mechanisms of resistance to BRAF inhibitors include the activation of MAPK-redundant signaling through the overexpression of receptor tyrosine kinases, resulting in AKT activation and RAS-CRAF-MEK signaling (33). To counteract these resistance mechanisms, a phase III trial is studying the efficacy of vemurafenib plus a MEK inhibitor versus vemurafenib alone (NCT01689519). Ongoing studies are also looking at using high dose IL-2 (NCT01683188); decitabine (NCT01876641); and bevacizumab (NCT01495988), a monoclonal antibody inhibitor of VEGF-A with vemurafenib in BRAF V600E positive patients.

Patients treated with vemurafenib have an increased incidence of squamous cell carcinoma and keratoacanthoma, especially in older patients with chronic sun damage. The increased incidence of squamous cell carcinoma in these patients has been shown to occur as a result of comorbid mutations in the *RAS* gene in patients that have a mutation in *BRAF*. Thus, concomitant treatment with a MEK inhibitor is also a means to counteract this side effect and reduce the frequency of squamous cell carcinomas in vemurafenib-treated patients (34).

IMMUNOTHERAPY

Systemic IL-2

In 1998, the FDA approved high-dose intravenous IL-2 as therapy for metastatic melanoma. IL-2 is a glycoprotein secreted by T helper cells. It promotes T-cell proliferation and the development of lymphokine-activated killer (LAK) cells, which have the ability to directly lyse tumor cells (Fig. 1) (35). Intravenous delivery of IL-2 produces an overall response rate of 16% and a complete response rate of 6% in patients with metastatic melanoma (36). However, due to IL-2-induced vascular

leak syndrome (VLS) and other associated toxicities, intravenous IL-2 therapy is limited to relatively healthy individuals. IL-2 is also currently being investigated in combination with anti-CTLA-4 therapy with lymphodepletion and adoptive cell transfer (NCT01701674).

Intralesional IL-2

To reduce systemic toxicity and increase local therapeutic effects, many groups have adopted an intralesional approach for IL-2 (see Table I) administration for treatment of cutaneous melanoma metastases. Three different clinical trials of intralesional IL-2 resulted in complete response rates of 62.5%, 40.7%, and 69% (37–39). Similarly, a case series of 39 patients treated with intralesional IL-2 reported a complete response rate of 76% (40), and another demonstrated a 100% response rate when 64 cutaneous and subcutaneous metastatic lesions in 3 patients were treated with biweekly administration of high-dose intralesional IL-2 in combination with imiquimod (41). Although these results are exciting, one concern is the possibility that intralesional IL-2 will expand regulatory T cells (Tregs). Tregs, an inhibitory T-cell population, are known to express the high affinity IL-2 receptor, CD25, which allows them to readily expand in response to IL-2 (42, 43). Thus, intralesional IL-2 may promote a systemic suppressive response by expanding Tregs. However, there are a few studies to suggest that patients treated with intralesional IL-2 have an increase in inflammatory T cells rather than Tregs and it has been suggested that these patients may live longer. Specifically, one study demonstrated an increased number of IFN-γ secreting T cells after administration of intralesional IL-2 (44) and a phase II clinical trial reported that there were no additional deaths from melanoma in patients who had survived for at least 25 months after initiation of intralesional IL-2 therapy, regardless of stage (38). These results are promising given that the reported 5-year survival for patients with cutaneous melanoma metastases is approximately 18% (2, 45). Additional studies will be needed to verify these results, especially since prior studies with intravenous IL-2 failed to demonstrate improvement in overall survival (46). We consider intralesional IL-2 regimens, especially in combination with topical imiquimod, to be a reasonable first line treatment for patients with cutaneous melanoma metastases. Ideally, future studies will compare these treatments head-to-head against surgical excision and/or systemic medications.

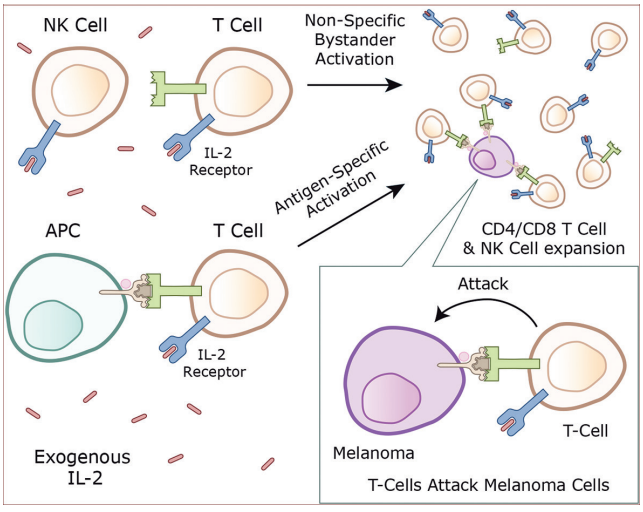

Fig. 1. Exogenous interleukin (IL)-2 promotes anti-melanoma immune responses. IL-2 is a cytokine usually produced by activated $CD4^+$ T cells. When used as a melanoma therapeutic, it is delivered either systemically or intralesionally. IL-2 promotes the proliferation, differentiation, and survival of $CD4^+$ and $CD8^+$ T cells as well as natural killer (NK) cells. The IL-2-induced expansion of these cells can occur in an antigen-specific or antigen-nonspecific fashion.

Imiquimod/toll-like receptor activation

Through activation of immune cells via the toll-like receptor 7 (TLR7)-MyD88-dependent signaling pathway, topical application of imiquimod induces the production of a variety of cytokines including IFN-α, TNF, and IL-12 (47, 48). This in turn contributes to the strong anti-tumor and anti-viral properties of this small molecule (49–51). Several neoplasms have been successfully treated with imiquimod: basal cell carcinoma, squamous cell carcinoma, extramammary Paget's disease, lymphoma, and melanoma (37, 49, 50, 52–58). However, some tumors are resistant to imiquimod therapy. It is well documented that subcutaneous and dermal melanomas are resistant to imiquimod (37, 59). The resistance mechanisms are multifactorial. Metastatic melanomas may develop resistance to the death receptor-independent apoptotic pathways induced by imiquimod (60). Secondly, poor drug penetration may limit the activation of plasmacytoid dendritic cells, which are normally recruited and activated in response to topical imiquimod (61–63). Finally, some melanocytic neoplasms such as dysplastic nevi seem to be uniformly resistant to imiquimod therapy (61, 64). Given these resistance mechanisms, we do not recommend imiquimod as a monotherapy option for treatment of cutaneous metastases.

Bacillus Calmette-Guérin

BCG is an attenuated live bovine tuberculosis bacillus that is used as a vaccination for human tuberculosis. BCG has also been employed to treat a variety of different malignancies. Initial attempts using BCG to treat melanoma showed some promise, but ultimately, enthusiasm for this modality decreased due to poor efficacy and the risk of death due to anaphylactic shock and development of infectious granulomas at sites of injection (65, 66). However, interest in BCG is again

on the rise given a number of reports identifying an inverse correlation between the incidence of cancer and the frequency of early life vaccinations with BCG or vaccinia in one retrospective analysis (67). In these studies, vaccinated patients experienced a 50% reduction in melanoma mortality risk over a study period of 5 years (67, 68). An inverse correlation was also noted for a history of BCG or vaccinia vaccination and Breslow's thickness, which is an important prognostic marker (67). The mechanism for this therapeutic benefit is unknown but it is interesting to note that both BCG and vaccinia, have sequence homologies with the HERV-K-MEL antigen, a product of a pseudo-gene closely related to endogenous retroviral genes (68).

Current clinical studies are evaluating the efficacy of different BCG-based treatment strategies for metastatic melanoma. For example, intralesional administration of BCG followed by intravenous infusion of ipilimumab in patients with stage III and stage IV melanoma is currently under investigation (NCT01838200). Other studies are evaluating treatment with CSF470 (a vaccine consisting of 4 lethally irradiated melanoma cell lines) plus BCG and molgramostin (rHuGM-CSF) for stage II and III melanoma (NCT01729663).

Interferon therapy

IFN-α is a type 1 interferon endogenously produced by macrophages, T cells, and natural killer cells that has been shown to have anti-tumor properties (69, 70). In humans, the benefit of IFN-α in the treatment of metastatic melanoma is controversial as the data on survival outcomes have been largely inconsistent between different trials. Initial meta-analyses of randomized control trials reported that IFN-α therapy is associated with a statistically significant improvement in disease-free survival but not in overall survival (71, 72). However, in a later randomized controlled trial, 444 patients who had undergone complete lymph node dissection were randomized to receive either adjuvant subcutaneous IFN-α 2a (3 MU) 3 times weekly or observation alone; the IFN therapy resulted in significantly improved disease-free survival (39% versus 27% at 4 year follow-up) and overall survival (59% versus 42% at 4 year follow-up) compared to observation (73). In another large randomized controlled trial, 1,256 patients with resected stage III melanoma were randomized to pegylated IFN-α 2b or observation for a median treatment duration of 12 months (74). This group reported a recurrence-free survival advantage in the IFN group (45.5% versus 38.9% over 4 years) but no difference in overall survival between the groups (74). Finally, in a meta-analysis of 14 randomized controlled trials, Mocellin et al. (75) reported significantly improved disease-free survival (18% risk reduction) and overall survival (11% risk reduction) in patients receiving IFN therapy. Despite the inconsistencies in overall survival outcomes, it does appear that adjuvant IFN-α therapy has a disease-free survival benefit in certain patients. More recent studies have indicated that both ulceration and tumor stage are predictive of IFN efficacy (76). However, treatment with IFN-α is also associated with significant toxicities. Many patients in the treatment groups experience severe fatigue, depression, and hepatotoxicity. In one study, pegylated IFN had to be discontinued due to toxicity in 31% of the patients (74).

With regards to treating cutaneous disease, there are several published case reports of intralesional IFN-α successfully treating melanoma *in situ*, either primary or recurrent (77–79). There are also published case reports of cutaneous metastases of melanoma as well as anorectal and esophageal melanoma responding to intralesional injections of IFN-β (80–84).

Cancer vaccines

Cancer vaccines attempt to activate the immune system to recognize and destroy cancer cells. Both autologous and allogenic vaccination strategies have been employed but response rates have been usually low ranging from 10 to 20%. These vaccines can be univalent or polyvalent in design. Univalent vaccines stimulate the immune system to respond against one specific antigen or carbohydrate moiety. Polyvalent vaccines allow the host to mount an immune response against multiple tumor antigens. Polyvalent strategies may incorporate allogenic whole cells, autologous tumor cells, shed tumor antigens, recombinant proteins, or tumor lysates (85).

Peptides derived from the melanoma antigens MART-1, Melan-A, gp100, and tyrosinase have all been employed in cancer vaccines. Vaccination with the gp100:209–217(210M) peptide resulted in high levels of circulating T cells which could recognize and kill melanoma cancer cells *in vitro* (86). In a single-group, phase II study, patients with metastatic melanoma were immunized with a gp100:209–217(210M) peptide vaccine in Montanide ISA-51 (incomplete Freund's adjuvant), followed by high-dose IL-2. This resulted in an objective clinical response in 42% of patients (87).

Schwartzentruber et al. (86) conducted a randomized, phase III trial involving 185 patients with stage IV or locally advanced stage III cutaneous melanoma. Patients were randomly assigned to receive IL-2 alone or gp100:209–217(210M) plus Montanide ISA-51 once per cycle, followed by IL-2. The vaccine–IL-2 group, as compared with the IL-2-only group, had a significant improvement in overall clinical response (16% versus 6%, $p=0.03$), as well as longer progression-free survival (2.2 months versus 1.6 month $p=0.008$). The median overall survival in the vaccine–IL-2 group was longer than in the IL-2–only group (17.8 months versus 11.1 months $p=0.06$). Thus, combining melanoma vaccines with IL-2 seems to add a modest benefit over IL-2 alone.

Carreno and colleagues from Washington University in St. Louis (88) investigated the role of IL-12p70 in melanoma patients by developing a vaccine with CD40L/IFN-γ –matured, IL-12p70–producing dendritic cells. Of the 7 patients treated with the vaccine, 6 developed sustained immunity against 3 separate gp100 melanoma antigens but only 3 of the 6 had a clinical response (1 complete remission and 2 partial responses). The production of IL-12p70 positively correlated with the development of antigen-specific CD8 T cells. Non-responders were seen to have lower IL-12p70 concentrations. Of note, non-responders were found to have a defect in IL-12p35 transcription, which led to decreased IL-12p70 production.

Oncolytic vaccines

Intralesional therapy for solid tumors has certain theoretical advantages over intravenous immunotherapy. For example, designing a melanoma-specific vaccine or engineering a T-cell adoptive transfer strategy will be difficult without specific knowledge of the tumor-derived determinants expressed by a particular melanoma. Unfortunately, MHC haplotypes alone cannot predict what antigens will be presented by a tumor because HLA-matched individuals may still respond to different antigenic determinants due to subtle differences in their antigen processing machinery (89). With intralesional immunotherapy, advanced knowledge of the melanoma antigens or the T-cell determinant structure is not required. Theoretically, the intralesionally-administered immunotherapy will induce a systemic immune response directed against the naturally processed and presented tumor antigens. For this reason, intralesional therapy holds great promise for the treatment of metastatic melanoma, especially cutaneous metastases.

In theory any systemic cancer vaccine can also be administered intralesionally. However, one oncolytic vaccine was specifically designed for the intralesional route. Talimogene laherparepvec (Oncovex or T-Vec) is a herpes simplex virus genetically engineered to express GM-CSF. Since it lacks ICP 34.5 and ICP 47, the virus prefers to grow in malignant cells. Thus, when administered intralesionally, it will specifically lyse tumors cells and create a high local concentrations of GM-CSF (90–92). The GM-CSF then attracts dendritic cells, which in turn process the remnants of dying melanoma cells and present these tumor antigens to T cells. In a phase II trial, Oncovex vaccination produced an objective clinical response rate of 26% in patients with stage IIIC or IV melanoma (93). Complete responses were not observed. Phase III trials are currently in progress (94).

One aspect of oncolytic viral therapy that is very encouraging is that responses have been documented at sites distant from the injection sites, a phenomenon known as an abscopal effect. As mentioned earlier, abscopal responses are sometimes seen in radiotherapy for cancer where a specific tumor site is treated and distant responses to untreated sites are observed. The fact that abscopal responses are being observed with oncolytic viral therapy confirms a dual action, namely a direct tumor lysing action at the site of injection and a systemic anti-cancer immune response capable of acting at non-injected sites.

Adoptive cell therapy

Early studies in murine models demonstrated the presence of lymphocytes in transplantable murine tumors (95–98). Subsequent studies showed that these "tumor infiltrating lymphocytes" (TIL) had potent anti-tumor activity when expanded *ex vivo* and reintroduced into tumor bearing hosts, a strategy used to treat lung and liver metastases (95–99).

In humans, adoptive cell therapies (Table I) utilizing TILs in combination with IL-2 have been promising. In one study, 86 patients with metastatic melanoma received autologous TILs plus high-dose intravenous IL-2. This resulted in an overall objective response rate of 31% with a complete response rate of only 5.8% (100). Lymphodepletion prior to TIL infusion has been employed in an effort to improve the *in vivo* microenvironment by reducing competition for growth factors and cytokines. Elimination of the native lymphocytes also opens up considerable "space" for the adoptively transferred cells, which can undergo homeostatic expansion to fill the vacated real estate. In one study, lymphodepletion with cyclophosphamide and fludarabine prior to infusing TILs resulted in an overall response rate of 51% (101). However, the complete response rate did not exceed 10% (101). In a follow-up study, metastatic melanoma patients received total body irradiation (2 or 12 gy) in addition to lymphodepletion with chemotherapy prior to TIL infusion. This resulted in an objective response rate of 52% and 72% and a complete response rate of 22%, the majority of which remained disease free at 3-year follow-up (102). Thus, lymphodepletion has a positive effect on adoptive cell transfer therapy.

Although TILs have not been studied extensively in patients with cutaneous metastatic melanoma, adoptive transfer of TILs may be a reasonable option for refractory cutaneous lesions. It is an exciting possibility because cutaneous metastases are easily accessible to surgical sampling and TIL harvesting. However, several limitations still exist with this technique. For one, it is very difficult to generate sufficient quantities of tumor-specific lymphocytes that can maintain their tumor-killing activity *in vivo*. Studies have suggested that less than 50% of melanomas will have TILs of sufficient potency (102). In addition, not all TILs are tumor reactive and selective expansion of tumor-specific clones without the concomitant expansion of bystander T cells remains challenging, labor intensive, expensive, and technically difficult.

Other methods are being studied in an attempt to overcome the barriers associated with TIL therapy. For example, T cells can be genetically engineered to express melanoma-specific TCRs. In one example, a retrovirus was used to transduce peripheral blood lymphocytes (PBL) to express a MART-1-specific TCR (Fig. S1[1]). Adoptive transfer of these cells induced a complete response in 2 out of 13 patients with MART-1-expressing melanomas (103).

In summary, current evidence suggests that immunotherapy with adoptive transfer of TILs is a promising avenue to treat patients with refractory metastatic melanomas. However, it is a technically challenging process and until melanoma-reactive lymphocytes can be efficiently manufactured, transferred, and maintained *in vivo* with their anti-tumor properties intact, it will be difficult to implement these therapies into routine clinical practice.

Anti-CTLA4 therapy

CTLA-4 is a transmembrane inhibitory receptor expressed on activated T lymphocytes (104–106). Upon binding to B7.1 or B7.2, CTLA-4 down-regulates T cell activation by inducing cell cycle arrest, inhibiting IL-2 secretion, and by down regulating T-cell cytokine receptors (105, 107–110). Due to its T-cell inhibitory effects, CTLA-4 blockade with anti-CTLA-4 monoclonal antibodies (see Table I) allows for unopposed T-cell activation, breaking of tolerance to tumor antigens, and ultimately tumor lysis (Fig. 2) (109).

Tremelimumab, a human monoclonal IgG2 anti-CTLA-4 antibody, was the first anti-CTLA-4 agent to be investigated in patients with metastatic melanoma (104). In phase I/II clinical trials, tremelimumab demonstrated antitumor activity in select stage III/IV melanoma patients(105). Although initial data was promising, a phase III study was later halted after tremelimumab failed to show any benefit over chemotherapy (111).

Despite tremelimumab's demise, ipilimumab, a human monoclonal IgG1 anti-CTLA-4 antibody, has been shown to benefit patients with metastatic melanoma in multiple clinical trials. In a randomized, double-blind, phase III study, 676 previously treated patients with unresectable stage III and IV melanoma received either ipilimumab monotherapy, ipilimumab in combination with gp100 peptide vaccine, or gp100 peptide vaccine monotherapy. They were then followed for up to 55 months (112). Median survival was significantly higher in the ipilimumab monotherapy arm (10.1 months) and in patients receiving ipilimumab in combination with gp100 (10.0 months) compared to gp100 monotherapy (6.1 months). There was no significant difference between ipilimumab monotherapy and ipilimumab in combination with gp100.

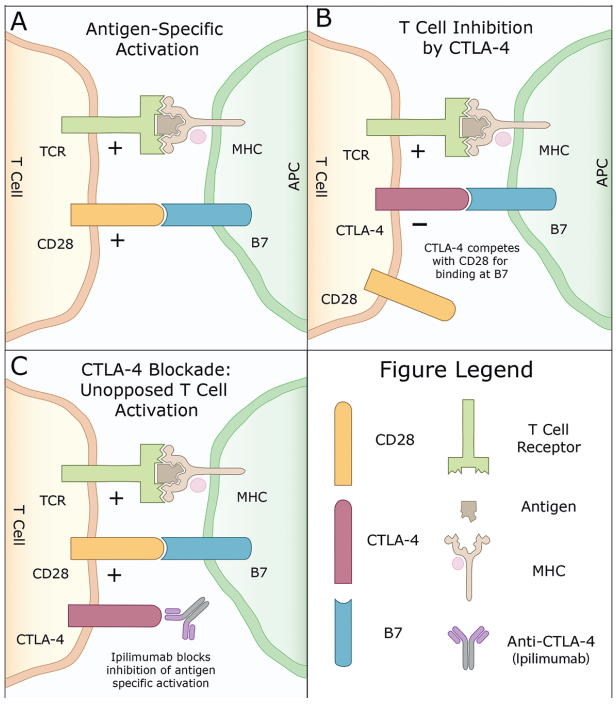

Fig. 2. Cytotoxic T-lymphocyte antigen (CTLA)-4 blockade supports unopposed T cell activation. A) For optimal T-cell activation the naïve T cell needs to receive two signals. T-cell receptor (TCR) recognition of its cognate presented in the context of an MHC molecule delivers the first signal. Not depicted here are the associated molecules CD3 and CD4 (or CD8, depending on the type of T cell), which are important for signal transduction and antigen recognition, respectively. Signal two is then provided by the CD28-B7 interaction. Once the T cell has received both signals, it then becomes activated. B) Following T-cell activation, CTLA-4-outcompetes CD28 for B7 binding. This competition allows T-cell activation to be attenuated as CTLA-4 transduces a negative signal to the T cells. C) Treatment with ipilimumab (anti-CTLA-4) blocks the negative signal that is usually delivered by CTLA-4. This allows CD28 to continuously interact with B7 and send a positive signal to the T cells, resulting in increased T-cell proliferation and promoting the generation of effector T cells.

In a separate phase III study, 502 patients with untreated stage III/IV melanoma were randomized to receive either ipilimumab in combination with dacarbazine or dacarbazine monotherapy with overall survival as the primary outcome measure (113). In this study, the ipilimumab and dacarbazine group demonstrated an increased overall survival (11.2 months) when compared to dacarbazine monotherapy (9.1 months). Overall survival rates at 12 months were 47.3% and 36.3% respectively (113). In a phase II trial, 75 patients with stage IIIc/IV melanoma status post resection were treated with ipilimumab. Significant immune-related adverse events (colitis, hypophysitis), defined as grade II, III, or IV, were seen in 37% of the patients, but they correlated with a longer relapse-free survival (114).

In a small percentage of patients, ipilimumab has induced a complete response; in some patients, up to 99 months has been reported (115), although the overall survival benefit in most patients has been modest. The

[1]http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-2035

ipilimumab clinical trials have, however demonstrated that utilizing pharmacologic agents to modulate the immune system can be an effective strategy to control melanoma progression. In BRAFV600E negative patients, it may be reasonable to treat non-resectable cutaneous melanoma metastases with ipilimumab. A phase I clinical trial is currently underway studying the use of intralesional ipilimumab combined with IL-2 for cutaneous metastases of melanoma (NCT01672450).

Anti-PD-1

The programmed cell death 1 receptor (PD-1), expressed by T cells, has two primary ligands; PD-L1, found on cancer cells and tumor-infiltrative macrophages; and PD-L2, found on antigen-presenting cells. When bound to PD-L1, PD-1 acts as a negative regulator of T cells. As with anti-CTLA-4 therapy, antibodies against both PD-L1 and PD-1 (Table I) have been developed to inhibit this down-regulatory pathway, allowing for unopposed T-cell activation (Fig. S2[1]). Ideally this will lead to activation of tumor-specific T cells and "bystander" T cells that may also contribute to the anti-cancer response. Recent phase I trials of nivolumab (anti-PD-1) in combination with ipilimumab (anti-CTLA4) and BMS936559 (anti-PD-L1) showed promise in treating patients with advanced melanoma (Fig. 3) (116). A phase 3 trial is currently ongoing comparing nivolumab, ipilimumab and a combination of nivolumab/ipilimumab (NCT01844505).

A second anti-PD-1 agent, pembrolizumab (formerly known as lambrolizumab) just recently was awarded FDA approval following data from an international multicenter, open-label, randomized, dose-comparative phase 1 study randomizing 173 patients with unresectable or metastatic melanoma, refractory to ipilimumab to receive pembrolizumab 2 mg/kg or 10 mg/kg intravenously once every 3 weeks. Overall response rate was achieved in 26% in both treatment arms. Adverse drug reactions included fatigue, rash and pruritus and no drug-related deaths were reported (117).

In an earlier published study, pembrolizumab was tested in 135 advanced melanoma patients, producing a response rate of 38%, with higher response rates seen in patients receiving a larger dose (10 mg/kg of body weight every 2 or 3 weeks compared to 2 mg/kg every 3 weeks). The median progression-free survival rate was greater than 7 months. Safety profile was similar to that of the most recent study (118). Combination therapies utilizing multiple immune modulating agents are showing great promise and will likely be common place in future treatment algorithms.

CONCLUDING REMARKS

The past decade has given rise to a variety of targeted therapies that hold great promise for the treatment of melanoma. Kinase inhibitors, immune activators, and a variety of combinations thereof are slowly increasing survival of these patients. Cutaneous metastases of melanoma provide a unique opportunity to evaluate the efficacy of traditionally systemic therapies as novel intralesional treatments. Thus, this patient population is ideal to more rapidly test creative strategies, which may lead to improved survival.

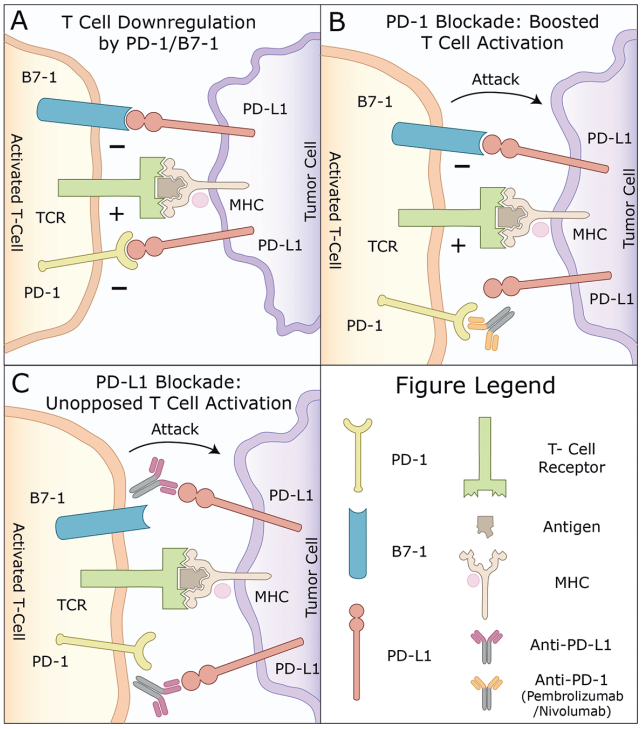

Fig. 3. Programmed cell death 1 receptor (PD-1) and programmed death-ligand 1 agents (PD-L1) promote T-cell killing of malignant melanoma. A) Similar to the way PD-L1-expressing antigen presenting cells can down-regulate T cells, malignant melanoma cells have a variety of mechanisms to prevent tumor-infiltrating T cells from mounting a cytotoxic anti-tumor immune response. One of these mechanisms is to express PD-L1, which has the ability to down regulate T cells by binding to PD-1 and B7-1 on their cell surface. B) PD-1 blockade with Pembrolizumab or Nivolumab boosts T-cell activation by preventing PD-1-mediated T-cell inhibition. C) PD-L1 blockage has the theoretical advantage in that it can block both the B7-1 and PD-1-mediated inhibition of activated T cells.

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