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Immunotherapy for Advanced Melanoma

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Abstract

Immunotherapy for melanoma has undergone significant change since the first attempts to treat patients with high dose IL-2. Herein, strategies to boost patient antitumor immunity through vaccination, treatment with agents that augment host immunity, and adoptive cell transfer will be discussed. The first two strategies have yielded only limited clinical success, but adoptive cell transfer therapy, particularly following a lymphodepleting, preconditioning regimen has resulted in objective response rates approaching 50%. For a number of reasons, lymphodepletion appears to be critical for maintenance of circulating antitumor T cells following adoptive cell transfer. Balancing antitumor efficacy, autoimmunity, and reconstitution of a functioning immune system remain challenging and potentially life-threatening issues.

Introduction

Melanoma is among the most immunogenic of all solid cancers, as supported by the phenomenon of spontaneous regression of primary tumors, which is seen in 3–15% of melanomas with unknown primaries (Morton et al., 1991). Moreover, the presence of tumor antigen-specific antibodies and tumor-specific cytotoxic T cells in the peripheral blood of melanoma patients has been well established (Lee et al., 1999). The ability of T lymphocytes, especially CD8 T cells, to prevent tumor formation has been shown in mice and humans (Shankaran et al., 2001; Chiao and Krown, 2003), and recent evidence indicates that the presence of infiltrating CD8 T cells within tumors is positively correlated with better prognosis in cutaneous melanoma (Ladanyi et al., 2007) as well as in several other types of cancers (Zhang et al., 2003). Thus, melanoma has been of interest as an intensively studied target for immunotherapy for over two decades.

In general, an effective antitumor CD8 T-cell response must fulfill a number of criteria. First, sufficient numbers of antitumor specific CD8 cells need to be generated *in vivo* or expanded *ex vivo*. Second, the CD8 T cells that are generated should be able to traffic and infiltrate into tumors. Finally, the CD8 T cells must be sufficiently activated within tumors such that they kill tumor cells, leading to tumor necrosis and/or tumor regression. The development of

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CONFLICT OF INTEREST

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murine models of melanoma, particularly those deploying transgenic T cells specific for melanoma antigens, has made it possible to better understand mechanisms underlying effective regression of established melanoma tumors and to make adjustments to improve immunotherapy prior to testing new strategies in the clinic (Finkelstein et al., 2004).

The most successful murine studies, however, do not always translate to safe, effective melanoma therapy for patients. Thus, we have attempted to list some of the most important clinical trials involving treatment of advanced melanoma that have been performed in the past few years (Table 1). The definition of clinical responses used in this review generally refers to "objective responses" as defined by World Health Organization (WHO) or WHO-RECIST criteria (James et al., 1999). The RECIST criteria include unidimensional measures of specific tumors and/or metastases with complete response (CR), partial response (PR), and progressive disease responses defined by a complete disappearance of all known lesions (CR), at least 30% decrease in size (PR), or 20% increase in the size of tumors (progressive disease). Patients who do not meet the criteria for PR or progressive disease are considered to have stable disease. Although RECIST criteria allow better comparison of clinical benefit between various trials, some authors have argued that "stable disease" rather than PR and CR may be a more realistic goal for newer biological agents and perhaps immunotherapy (Michaelis and Ratain, 2006).

Currently, there are three major approaches to boost antitumor CD8 T-cell responses in patients with melanoma as summarized in Figure 1: (1) non-specific stimulation of antitumor immune responses by stimulating endogenous effector cells with cytokines or removing inhibitory signals for T-cell activation, (2) active immunization (that is, vaccines) to enhance endogenous antitumor responses *in vivo*, and (3) adoptive cell-transfer (ACT) therapy as exemplified by *ex vivo* selection and expansion of autologous antitumor-specific CD8 T cells that are subsequently transferred back to the patient. This review will discuss each of these therapeutic strategies in terms of the preclinical studies that underlie their mechanisms, their reported efficacy in melanoma clinical trials, as well as their distinct toxicity profiles.

Nonspecific stimulation of antitumor immune responses

IFN-α and IL-2 therapy.

In the 1980s, IFN-α was reported to inhibit the growth of B16 murine melanoma cells *in vitro* and *in vivo* (Bart et al., 1980). Kirkwood et al. (1996) demonstrated for the first time that high-dose IFN-α2b not only prolongs disease-free survival, but also overall survival in high-risk patients with melanomas thicker than 4 mm or with lymph node metastasis.

To date, IFN-α has been one of the most intensely investigated immunotherapeutic agents for melanoma, and it is the only currently approved adjuvant therapy for the treatment of high-risk melanoma patients. The effect of IFN-α2b on disease-free survival was confirmed in a subsequent trial, but the study failed to prove significant effect on overall survival (Kirkwood et al., 2000). In an extensive meta-analysis, investigators concluded that adjuvant treatment with IFN-α reduces the incidence of melanoma recurrence by ~26% and provides benefit in overall survival (15% reduction in risk of death) that is not quite statistically

significant ($P = 0.06$) (Wheatley et al., 2003). The use of polyethylene glycol-conjugated IFN, which has a substantially longer half-life than the unconjugated molecule, resulted in significant clinical responses of ~33% when used in combination with temozolomide in patients with advanced disease (Hwu et al., 2006). Polyethylene glycol-conjugated IFN may also have utility in the adjuvant setting in melanoma patients with a high risk of recurrence (Eggermont et al. *j Clin Oncol*, 2007 ASCO Annual Meeting Proceedings Part I. vol. 25, no. 18S (June 20 Supplement); 2007: 8504). Considering its efficacy and ease of administration, polyethylene glycol-conjugated IFN is worth further investigation to define its place in the treatment of patients with advanced stage melanoma.

IL-2 is known for its potent ability to activate CD8 T cells and natural killer cells, resulting in development of the so-called lymphokine-activated killer cells. Because systemic administration of high dose of IL-2 resulted in regression of established pulmonary metastases in B16 melanoma-bearing mice (Rosenberg et al., 1985b), Rosenberg *et al.* performed the first clinical trial using autologous lymphokine-activated killer cells and systemic IL-2 in patients with metastatic melanoma. Although initial clinical results were promising (Rosenberg et al., 1985a), subsequent trials demonstrated that high-dose IL-2 provided consistent, but low, overall response rate of ~ 13–17% (7–9% PR and 6–8% CR) (Rosenberg et al., 1994a; Atkins et al., 1999). Currently, IL-2 is the only FDA-approved immunotherapeutic agent for treatment of patients with metastatic melanoma. Notably, within the 15 years of follow-up in these studies, >80% of the complete responders to high-dose IL-2 treatment have not had recurrences and may be considered cured (personal communication, SA Rosenberg, NCI).

The modest benefits of IL-2-based treatment regimens, however, must be weighed against the serious adverse side effects and the high cost of therapy. Moreover, recent studies have shown that IL-2 expands $CD4^+$ $CD25^+$ $Foxp3^+$ -regulatory T cells (Tregs) *in vivo*, thereby possibly inducing immune tolerance (Malek and Bayer, 2004). Furthermore, administration of high-dose IL-2 resulted in a nearly fourfold increase in the frequency of $CD4^+$ $Foxp3^+$ Tregs in the peripheral blood when compared with pretreatment levels (Ahmadzadeh and Rosenberg, 2006), suggesting that Tregs may play important downstream roles in determining responses to IL-2.

Several studies have been conducted to study combinations of IL-2 and chemotherapy in advanced melanoma in an effort to improve response rates and survival. Unfortunately, recent phase-III trials of chemotherapy with high-dose IL-2 administration resulted in increased serious toxicity to patients and failed to confirm significantly better durable response rates (or overall survival) compared with chemotherapy alone (Rosenberg et al., 1999; Ridolfi et al., 2002; Keilholz et al., 2005).

Anti-CTLA-4 therapy.—Optimal T-cell activation requires signaling through both the T-cell receptor (TCR) and the costimulatory receptor CD28, which is constitutively expressed on T cells. Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), a member of CD28 immunoglobulin family, is an inhibitory receptor expressed mainly by activated T cells and Tregs. CTLA-4 binds CD80 or CD86 with higher affinity and avidity than does CD28, thereby inhibiting CD28-dependent T-cell activation and decreasing IL-2 production (Teft et

al., 2006). The critical negative regulatory function of CTLA-4 was established upon characterization of CTLA-4-deficient mice, which exhibited profound lymphopro-liferation and autoimmunity.

mAbs targeting CTLA-4 have been shown to increase host antitumor immunity in a number of tumor models (Leach et al., 1996). However, anti-CTLA4 mAb alone failed to produce similar antitumor efficacy in a B16 melanoma model, even when the treatment was initiated at the time of tumor inoculation (van Elsas et al., 1999). By contrast, a short course of anti-CTLA-4 blocking antibody in combination with a GM-CSF-expressing irradiated tumor cell vaccine eradicated established B16 melanoma in 80% of wild-type mice and protected those mice from subsequent tumor challenge (van Elsas et al., 1999). When tumors were successfully rejected, depigmentation was observed in 50% of the treated mice. Since depigmentation, a marker of autoimmunity, is an uncommon event following vaccination alone and has not been observed with anti-CTLA-4 monotherapy, these findings suggested that CTLA-4 played a significant role in breaking peripheral tolerance.

Fully humanized antibodies against human CTLA-4, MDX-010 (ipilimumab), and CP-675,206 (ticilimumab) have been evaluated in several published phase-I and phase-II trials (Table 1). Anti-CTLA-4 monotherapy has yielded response rates of 7–15% in heavily pretreated patients with metastatic melanoma (Korman et al., 2005; Ribas et al., 2005). Administration of MDX-010 in conjunction with peptide vaccination to patients with stage-IV melanoma resulted in objective cancer regression in 3 of 14 patients (two CRs and one PR) and grade-III/IV autoimmune manifestations in 6 of 14 patients (43%) (Phan et al., 2003). Combination therapy with anti-CTLA-4 and IL-2 in patients with metastatic melanoma yielded a similar 22% response rate coupled with a 14% rate of grade-II/IV autoimmune toxicities (described below). Given an anticipated 15% response rate to IL-2 alone in the treatment of metastatic melanoma, there does not seem to be a synergistic effect (Maker et al., 2005).

Treatment with anti-CTLA-4 mAb is frequently associated with adverse immune events, most commonly involving the skin and the gastrointestinal tract. Grade-III and grade-IV adverse immune events, especially enterocolitis (21%;Beck et al., 2006) and autoimmune hypophysitis (5%; Blansfield et al., 2005) have been reported. A dermatitis characterized by pruritic macules and papules occurred in 14% of stage-IV melanoma patients treated with ipilimumab as monotherapy (Jaber et al., 2006). The erythematous macules and papules present in anti-CTLA-4 dermatitis were photodistributed in some patients, and a Koebner-like phenomenon could be elicited in others (Figure 2). Peripheral eosinophil counts were increased by fourfold in patients who had experienced the eruption compared with in those who had not (Jaber et al., 2006). Of note, this pruritic skin eruption was similar in clinical and histological appearance (superficial perivascular lymphocytic infiltrate with frequent eosinophils) to the macular-papular rash most commonly associated with drugs such as penicillins (Jaber et al., 2006). Autoimmune side effects have been correlated with clinical antitumor responses and relapse-free survival (Attia et al., 2005), suggesting that antitumor immunity is achieved by blockade of CTLA-4 at the increased risk of autoimmunity in other tissues.

Toll-like receptor agonists.—Synthetic deoxycytidyl-deoxyguanosine oligodeoxynucleotides contain unmethylated CG motifs similar to those observed in bacterial DNA. By triggering Toll-like receptor-9 (TLR9), these oligodeoxynucleotides trigger an immunostimulatory pathway that includes dendritic cell (DC) activation and production of proin-flammatory cytokines such as type-I IFN, thus potentially favoring antitumor immunity. In humans, only plasma-cytoid DCs and B cells are known to express TLR9 (Krieg, 2004).

Because TLR9 agonists have shown efficacy in animal tumor models (Lonsdorf et al., 2003), they are being evaluated for their therapeutic potential in clinical trials for melanoma patients. The first human trial of a synthetic deoxycytidyl-deoxyguanosine oligodeoxynucleotides combined with a melanoma peptide showed that use of CpG 7909 as an adjuvant to Melan-A/Mart-1 peptide vaccination resulted in 10-fold increase in circulating Melan-A-specific CD8 T cells compared with use of peptide alone. Clinical response rates were not obtained in this phase-I study due to the short follow-up period (Speiser et al., 2005).

Imiquimod, a TLR7 agonist that is currently FDA-approved for the topical treatment of actinic keratosis, external genital warts, and superficial basal cell carcinoma, also appears to hold potential for the treatment of melanoma. Several cases of successful treatment with topical 5% imiquimod of otherwise untreatable cutaneous metastases of malignant melanoma (Steinmann et al., 2000) and melanoma *in situ* (Ray et al., 2005) have been reported. Therapeutic approaches based on TLR agonists may be of particular relevance in cases where standard therapies have been refused or are contraindicated.

Cancer vaccines

Peptide-based vaccination strategies.—The identification of defined tumor antigens that can be the targets of T cells has led to development of new immunotherapeutic strategies (Boon et al., 1997). Tumor antigens isolated from melanomas include MART-1/Melan-A, gp100, and tyrosinase, all of which are melanocytic differentiation antigens that are also expressed by normal melanocytes. Anti-melanoma-specific CTLs have been found in peripheral blood, draining lymph nodes, and within tumors in melanoma patients (Pittet et al., 1999; Zippelius et al., 2004). It has been suggested that these cells may be unable to eradicate invasive tumors due to low frequency and/or an immunosuppressive host microenvironment. Therefore, administration of cancer vaccines may boost endogenous antitumor immune responses *in vivo*.

Vaccines are classified as either univalent, stimulating the immune system against a particular antigen, or polyvalent, eliciting immune responses against multiple antigens. The most extensively studied melanoma vaccines are polyvalent whole cells or cell lysates derived from allogenic or autologous tumor cells. One advantage of this approach is that the vaccine immunizes the patient against a variety of tumor antigens, without attempting to predict which antigen will elicit the most effective antitumor responses. Since these vaccines are expected to be immunogenic in patients with various HLA types, they are potentially beneficial for a larger patient population.

Early trials of polyvalent whole-melanoma-cell vaccines (Mitchell et al., 1988; Morton et al., 1992; Hsueh et al., 2002) suggested vaccines could elicit immunological responses in melanoma patients and provide clinical benefit to some patients. However, later trials (Table 1) involving larger numbers of patients failed to show a clinical benefit (Sondak et al., 2002; Faries and Morton, 2005). One polyvalent whole-cell vaccine (Melacine®) combined with low-dose cyclophosphamide was shown to provide superior quality of life during therapy, although there was no demonstrable difference in response rates and survival compared with patients treated with a four-drug chemotherapy regimen (Mitchell, 1998).

Immunization with melanoma-associated peptide antigens is a strategy that has been vigorously pursued. In one of the first clinical trials using melanoma cell-derived peptides, Rosenberg et al. (1998a) vaccinated stage-IV melanoma patients with g209-2M, a modified immunodominant peptide of the gp100 antigen. On the basis of immunological assays, 91% of patients could be successfully immunized with this synthetic peptide, and 13 of 31 patients (42%) receiving the peptide vaccine plus IL-2 had objective cancer responses. All patients, however, eventually developed progressive disease. Nevertheless, this study provided essential proof-of-principle of the concept that immune responses against self-antigens can be elicited in patients with advanced melanoma. Although encouraging, this and subsequent clinical studies also highlighted the fact that tumor progression can occur despite induction of an antigen-specific immune response (Powell et al., 2006). Strategies using expression of melanoma peptides via recombinant adenoviral vectors (Rosenberg et al., 1998b) or with plasmid vectors encoding tumor antigen (Rosenberg et al., 2003) were initially attractive due to their low cost and ease of administration, but have not resulted in clear elicitation of immune or clinical responses in patients. Cancer-testis antigens, expressed in different tumors and normal testis, are also potential targets for melanoma immunotherapy. While an initial study of the cancer-testis antigen known as MAGE-3 resulted in tumor regression in some patients (Marchand et al., 1999), later studies with this cancer-testis antigen showed poor clinical and immunological responses (Kruit et al., 2005).

DC-based vaccination strategies.—Effective presentation of the defined tumor antigens to the immune system of a cancer patient remains to be one of the most important challenges in the field. The presentation of tumor antigen by DCs or other antigen-presenting cells is a central step in the induction of an antigen-specific T-cell response, and DCs, therefore, have been proposed to be an ideal tool for the induction and augmentation of an immune response in a vaccination setting (Grabbe et al., 1995). In recent years, the clinical use of DCs has been facilitated by development of techniques to generate large numbers of these cells *in vitro* from blood monocytes or CD34+ progenitor cells. The first human trial using monocyte-derived peptide- or tumor lysate-pulsed DCs for antigen delivery enrolled 16 patients with advanced melanoma and yielded five objective responses (ORs) (2/16 CR, 3/16 PR) (Nestle et al., 1998). Using a similar vaccination strategy with larger numbers of patients, however, Schadendorf et al. (2006) demonstrated only a 3.8% OR rate that was similar to the OR rate for dacarbazine. Other DC vaccination trials have not resulted in significant objective clinical responses, but have shown delayed-type hypersensitivity to the peptide antigen (Thurner et al., 1999) or a delay in tumor progression when patients were able to mount immune responses to multiple antigens used in the

multivalent DC-based vaccine (Banchereau et al., 2001). Thus, although immunological responses to selected vaccination strategies have often been detected, their clinical utility as single agents is not promising. In summarizing the results from 35 representative published vaccine studies, Rosenberg et al. (2004) calculated an OR rate of 3.8% (7.1% for DCs, 4.2% for modified tumor cells, 4.0% for peptide-based vaccines, and 0% for pox viruses) for cancer vaccines. Currently there are no vaccination strategies that consistently induce melanoma regression.

ACT therapy

CTL therapy.—In CTL therapy, peripheral blood mononuclear cells are isolated from melanoma patients and stimulated with autologous antigen-presenting cells pulsed with HLA-restricted peptide epitope of MART-1 or gp100. After several rounds of stimulation, single clones are selected *in vitro* for their abilities to specifically kill antigen-positive tumor targets. CTL clones are further expanded *in vitro* before being adoptively transferred back to patients (Ho et al., 2002). Early clinical trials with CTL therapy met with limited success as antigen-specific CD8 T cells persisted *in vivo* only for a short period of time. In addition, recurrent tumors were found to selectively lose the targeted antigen(s) (Yee et al., 2002).

Tumor-infiltrating lymphocyte (TIL) therapy.—In contrast to cancer vaccines, which activate the immune system *in situ*, TIL therapy relies upon (1) isolation and propagation of autologous T cells present in patient tumors (usually metastases) in the presence of high levels of IL-2, (2) selection of highly avid clones that produce high levels of IFN-γ against multiple melanoma cell lines, and (3) expansion of those cells *in vitro* with anti-CD3 and IL-2 to large numbers that are then transferred back to the patient (Dudley and Rosenberg, 2003).

Early attempts with TILs and high-dose IL-2 in melanoma patients achieved limited clinical success (Rosenberg et al., 1994b) and were generally characterized by poor persistence of TIL clones *in vivo* following adoptive transfer. Although cultured TIL clones often showed high avidity toward tumor-associated melanoma peptides, only rarely were clinical remissions achieved in excess of that expected for IL-2 therapy alone. Thus, high-avidity T-cell clones alone were not sufficient for treatment efficacy.

Remarkable results in this field were first reported by Rosenberg et al., who used non-myeloablative, lymphodepleting preconditioning followed by infusion of autologous TILs and IL-2 (Dudley et al., 2002a). Subsequent studies at the NCI confirmed response rates of ~50% in advanced melanoma patients with metastatic disease (Dudley et al., 2005). Several patients receiving treatment regimen achieved impressive clinical regression of large bulky tumors (figure 3). The rationale for this lymphodepleting preconditioning regimen was based on early animal studies demonstrating that depletion of endogenous lymphocytes by chemotherapy (prior to adoptive transfer of T cells) resulted in improved antitumor responses (Cheever et al., 1980; North, 1982; Dummer et al., 2002). The mechanisms by which lymphodepleting regimens improve outcome of ACT therapy have been shown to involve elimination of Tregs, increasing the availability of T-cell growth-promoting

cytokines, and improvement of the function and/ or availability of antigen-presenting cells as discussed below (Gattinoni et al., 2006).

Naturally occurring Tregs possess the ability to suppress or antagonize the function of other T cells. Tregs are crucial for maintenance of peripheral self-tolerance and suppression of antitumor immunity (Sakaguchi, 2005). The Tregs described in this review are characterized by expression of the forkhead box P3 (FoxP3) transcription factor, CD4, and activated T-cell markers, including CD25 (IL-2 receptor-a), glucocorticoid-induced tumor-necrosis factor-receptor family-regulated gene, and CTLA-4.

$CD4^+$ $CD25^{hi}Foxp3^+$ cells are relevant to immunotherapy for melanoma for a number of reasons. Co-transfer of $CD4^+$ $CD25^+$ Tregs reduced the ability of transgenic, antigen-specific CD8 T cells to induce B16 murine melanoma tumor regression *in vivo* (Antony et al., 2005). Clinically, $Foxp3^+$ Tregs were overrepresented in metastatic lymph nodes from patients with melanoma. Following isolation, these Tregs inhibited proliferation and cytokine production of tumor-infiltrating CD4 and CD8 cells *in vitro* (Viguier et al., 2004). In ovarian cancer patients, reduced survival was associated with increased tumor-infiltrating Treg levels (Curiel et al., 2004), whereas increased survival was associated with a high ratio of intratumoral CD8 T cells to Tregs (Sato et al., 2005). Even though the presence of Treg cells in tumor lesions has not been conclusively connected with progression of cancers, the suppressive function of Treg cells may contribute to poor clinical response in non-lymphodepleting patients receiving ACT therapy.

Lymphodepleting preconditioning regimens may also be effective because they reduce the size of the host endogenous lymphocyte pool, allowing newly transferred T cells access to growth promoting cytokines (Goldrath et al., 2000). Because the size of the T-cell pool in humans and mice is tightly maintained at a nearly constant level, adoptive transfer of cells into a lymphopenic host will result in rapid expansion of the newly transferred antigen-specific T cells in an IL-7- and IL-15-dependent manner (Tan et al., 2002). Dummer et al. (2002) first reported that the antitumor benefits of lymphodepletion were dependent on homeostatic expansion of a polyclonal T-cell population within lymph nodes, indicating that T cells can be induced to mount an effective autoimmune response against self-antigens when homeostatic expansion occurs at the time of antigen encounter. In the pmel-1 mouse model of ACT therapy for melanoma, lymphodepletion also enhanced antitumor efficacy of adoptively transferred T cells in an IL-7- and IL-15 dependent manner (Gattinoni et al., 2005a).

Preconditioning of the host with systemic chemotherapy or total-body irradiation before ACT therapy was initially thought to result in apoptosis or necrosis of tumor cells, allowing more efficient presentation of tumor antigens by host DCs to the adoptively transferred CD8 cells. Recent results, however, showed that total-body irradiation caused mucosal injury, resulting in microbial translocation from gastrointestinal tract and systemic release of lipopolysaccharide. Signaling through TLR4, lipopolysaccharide increased the absolute numbers of activated DCs, which subsequently secreted high levels of T-cell-activating cytokines (Paulos et al., 2007).

While lymphodepletion remarkably improves the outcome of ACT therapy, it is not the only factor that determines the clinical response. Preclinical and clinical studies have implied that the differentiation state of transferred CD8 T cells may be crucial for the success of ACT therapy. Upon encoun-ter with antigen, naïve CD8 T cells proliferate and differentiate through early, intermediate, and late effector stages depending on signal strength (Lanzavecchia and Sallusto, 2002). Differentiation from early stage to late stage of effector CD8 T cells is characterized by progressive downregulation of CD62L, CCR7, β7-integrin, and CD27 and concurrent upregulation of CD44, CD69, CD25, granzyme B, and perforin. After multiple rounds of *in vitro* stimulation with antigen and IL-2, activated pmel-1-transgenic CD8 T cells acquired terminally differen-tiated effector properties, including increased cytolytic activity and higher levels of IFN-γ production. How-ever, when adoptively transferred into lymphodepleted B16 tumor-bearing wild-type mice, terminally differentiated pmel-1 CD8 T cells were at least 100-fold less effective than early effector CD8 cells in antitumor efficacy (Gattinoni et al., 2005b). In addition, terminally differentiated pmel-1 CD8 T cells proliferated poorly *in vivo*, suggesting they might have already exhausted their abilities to proliferate and to persist *in vivo* once adoptively transferred. On the other hand, early effector CD8 T cells possessing essential adhesion molecules for trafficking to lymph nodes showed superior antitumor efficacy compared with T cells that could not traffic to lymph nodes (Gattinoni et al., 2005b). These findings suggest that trafficking of less differentiated CD8 T cells to lymph nodes (where they can be effectively stimulated by DCs) may be more effective in ACT for cancer.

These findings pose new challenges for ACT-based immunotherapy. Currently, the only criteria clinically used to screen for TIL clones is their ability to produce high levels of IFN-γ and to kill antigen-specific target cells *in vitro*. Selected TIL clones undergo several rounds of expansion with anti-CD3, IL-2, and allogeneic antigen-presenting cells, which inevitably led to selection of late-stage or terminal differentiated TIL clones for adoptive transfer. Two ACT clinical studies using tumor-reactive CD8 T cells generated and expanded *ex vivo* through multiple stimulations did not show substantial ORs, although transferred CD8 cells showed potent antitumor activity *in vitro* (Dudley et al., 2002b; Yee et al., 2002). Thus, balancing the quality and quantity of the transferred cells poses one of the greatest challenges for ACT therapy.

While lymphodepletion combined with ACT shows great promise, lymphodepletion appears to increase the risk of viral infections and virus-associated cancers, perhaps because of the long period of time required for lymphocyte recovery following chemotherapy (Dudley et al., 2002b). Furthermore, use of high-dose IL-2 following transfer of T cells exposes patients to many of the same risks incurred by IL-2 therapy in the past. The use of genetically engineered T cells that endogenously express IL-2 may remove the need to give patients high systemic doses of IL-2 (see below). Alternatively, modifications of IL-2 that result in more T-cell (vs natural killer cell) selectivity may reduce systemic toxicity (Shanafelt et al., 2000). Clearly, advances in reducing the use of systemic IL-2 (or replacing it with less toxic alternatives) would make ACT a more attractive (and safer) therapy for melanoma.

Additional strategies for improving immunotherapy for melanoma

Engineered T-cell therapy.

Although ACT therapy has achieved objective tumor regression in patients with metastatic melanoma, not all the biopsy specimens yield high-avidity TIL clones for adoptive transfer (Dudley et al., 2003). One approach to overcome this limitation is to generate and adoptively transfer engineered autologous T cells that express cloned high-affinity TCRs for melanoma-specific antigens. TILs recognize tumor-associated antigens through major histocompatibility complex-restricted TCRs that are composed of TCR-α and -β chains. Rosenberg et al. have identified individual patient TIL clones showing high affinity *in vitro* to specific melanoma antigens (for example, gp100); they cloned the TCR-α and -β chains from these TILs and subsequently expressed these TCRs using retroviruses in peripheral blood T cells from other patients (Morgan et al., 2003). The genetically engineered T cells secreted high levels of IFN-γ and were cytolytic against both melanoma cell lines and autologous melanoma cells. Importantly, following adoptive transfer into melanoma patients lymphodepleted by chemotherapy, transduced T cells expressing the cloned TCR chains persisted at high levels in the peripheral blood for at least 2 months (and up to 1 year) in patients (Morgan et al., 2006).

Although clinical response rate in this study was lower than that achieved using autologous TILs (Dudley et al., 2005), several approaches to increase efficiency of TCR expression may potentially enhance clinical efficacy (Cohen et al., 2007; Zhao et al., 2007). Furthermore, transducing tumor-reactive T cells with IL-2 (Liu and Rosenberg, 2001) or IL-15 (Klebanoff et al., 2004) may promote survival of TILs without subjecting patients to the toxicities of systemically administered IL-2.

Potential synergy of immunotherapy and blockade of chemokine receptor-mediated survival pathways

Clinical studies showed that selected chemokine receptors, particularly CXCR4, are often upregulated in a large number of common human cancers, including melanoma, and that chemokine receptors (in concert with their chemokine ligands) facilitate cancer survival and metastasis through a number of mechanisms (Muller et al., 2001; Murakami et al., 2002; Kakinuma and Hwang, 2006). The CXCR4 ligand, CXCL12, protected B16 cells from killing by activated pmel-1 CD8 T cells *in vitro* (Lee et al., 2006), presumably through activation of the phosphoinositide-3-kinase and its downstream effector, Akt (Murakami et al., 2003). Inhibition of CXCR4 by a peptide antagonist, T22, in combination with cyclophosphamide or anti-CTLA-4 antibody significantly reduced metastatic tumor burden in the lungs compared with treatment with cyclophosphamide or anti-CTLA-4 alone (Lee et al., 2006). This study suggests that pretreatment of patients with a chemo-kine-receptor antagonist prior to immunotherapy may result in better clinical responses.

Summary

Until recently, immunotherapy for melanoma has made only small incremental improvements since the first attempts to treat patients with high-dose IL-2. Vaccination

strategies alone have shown little efficacy, whereas attempts to boost the endogenous host antitumor response with agents such as anti-CTLA4 have met with marginal success. In the case of the latter agent, clinical efficacy in shrinking tumors has often come at a high price in terms of autoimmune toxicities that have been potentially life threatening. By contrast, ACT therapy, particularly following lymphodepleting, preconditioning regimen has resulted in response rates approaching 50%. Lymphodepletion appears to be particularly critical for the success of these therapies because (1) it provides access to cytokines that promote growth and survival of newly transferred T cells and (2) it removes suppressive or regulatory T-cell populations.

Developing safe lymphodepleting regimens will be a challenge, since toxicities of high-dose IL-2 and delays in immune reconstitution remain major impediments to ACT. Future enhancements to ACT include using total-body irradiation to activate antigen-presenting cells via TLRs (in addition to reducing endogenous T-cell populations through bone marrow suppression). These experiments suggest that the growing library of clinical-grade TLR agonists may prove to be valuable adjuvants for ACT. Lastly, genetic manipulation of either allogeneic or autologous T cells with highly avid cloned TCRs selected for clinical efficacy or with cloned cytokines such as IL-2 and IL-15 may allow production of highly tumor-reactive T cells that can be delivered far more quickly and with less cost than the current methods of culturing tumor-infiltrating T cells from resected tumors. While prognosis for the majority of patients with advanced metastatic melanoma remains relatively poor, almost half of the patients with advanced melanoma can obtain substantial ORs using current ACT regimens. Improvements to current treatment strategies that are now being refined in mouse models of melanoma suggest that future enhancements in clinical efficacy are forthcoming.

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Abbreviations:

ACT	adoptive cell transfer
CR	complete response
CTLA	cytotoxic T lymphocyte-associated antigen
DC	dendritic cell
OR	objective cyte
TLR	Toll-like receptor
Tregs	regulatory T cells

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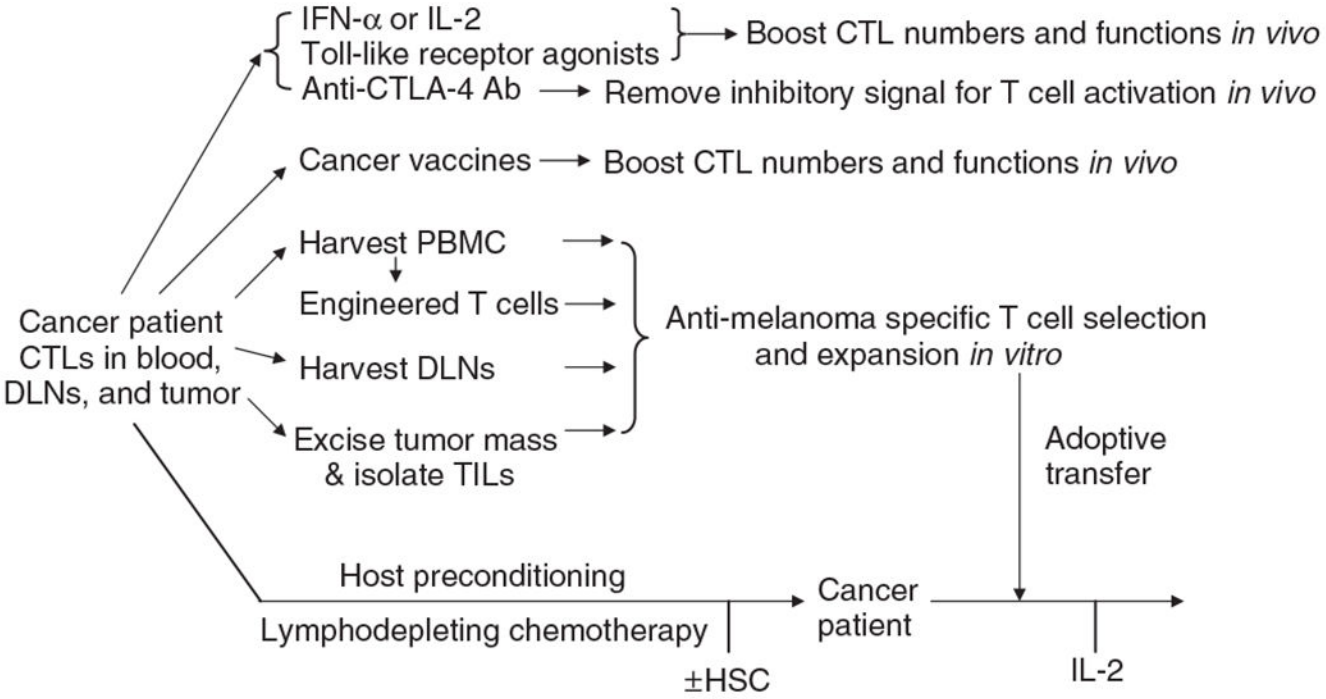

Figure 1. Strategy for immunotherapy of melanoma.

CTL, cytotoxic T lymphocyte; DLN, draining lymph node; PBMC, peripheral blood mononuclear cells; CTLA, cytotoxic T lymphocyte antigen; TIL, tumor-infiltrating lymphocyte; HSC, hematopoietic stem cell.

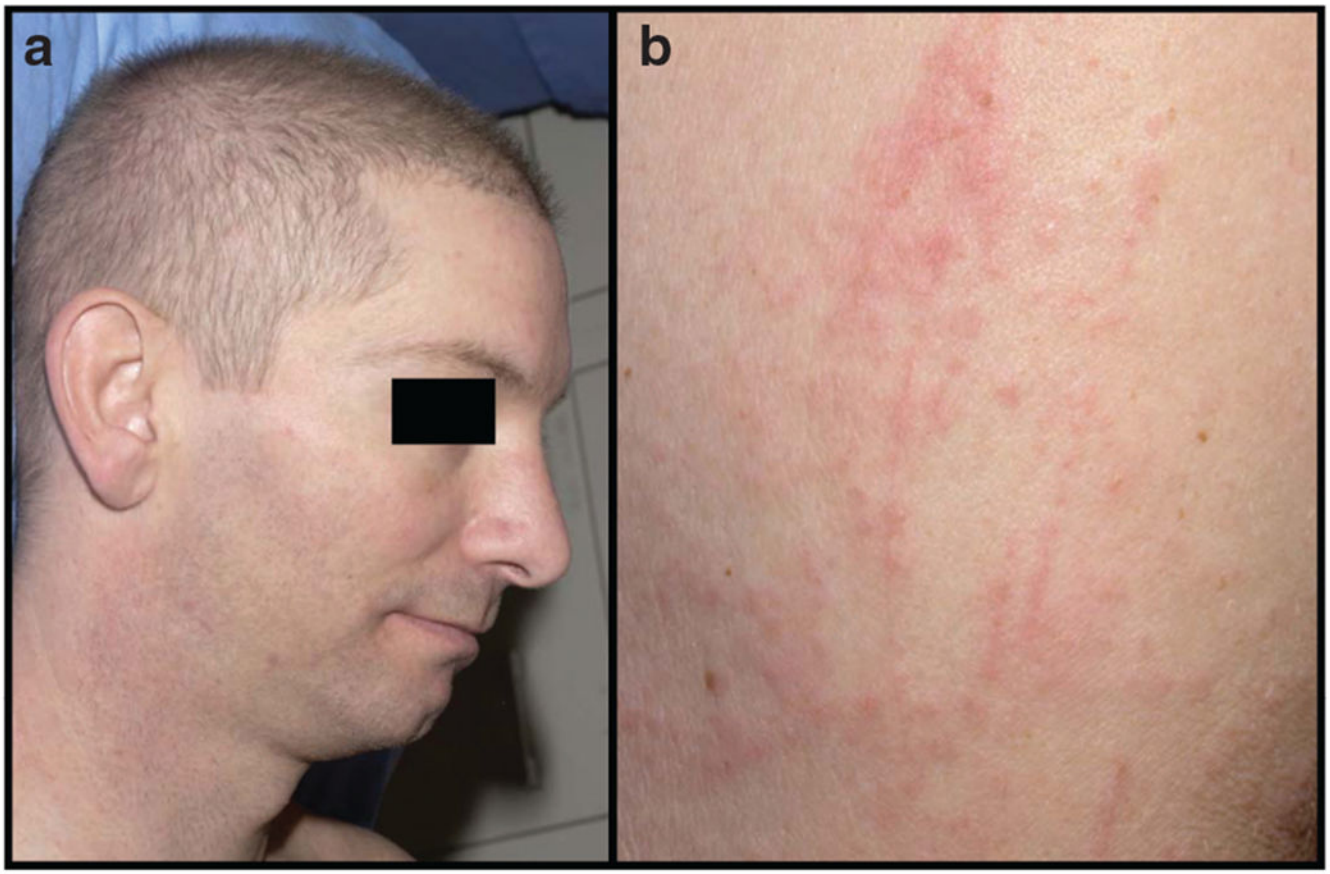

Figure 2. Skin dermatitis following treatment with anti-CTLA-4 mAb as a single agent as described in Jaber et al. (2006).

(**a**) Photodistributed macules and papules on face and neck, and (**b**) erythematous papules with Koebner-like phenomenon elicited by the trauma of scratching.

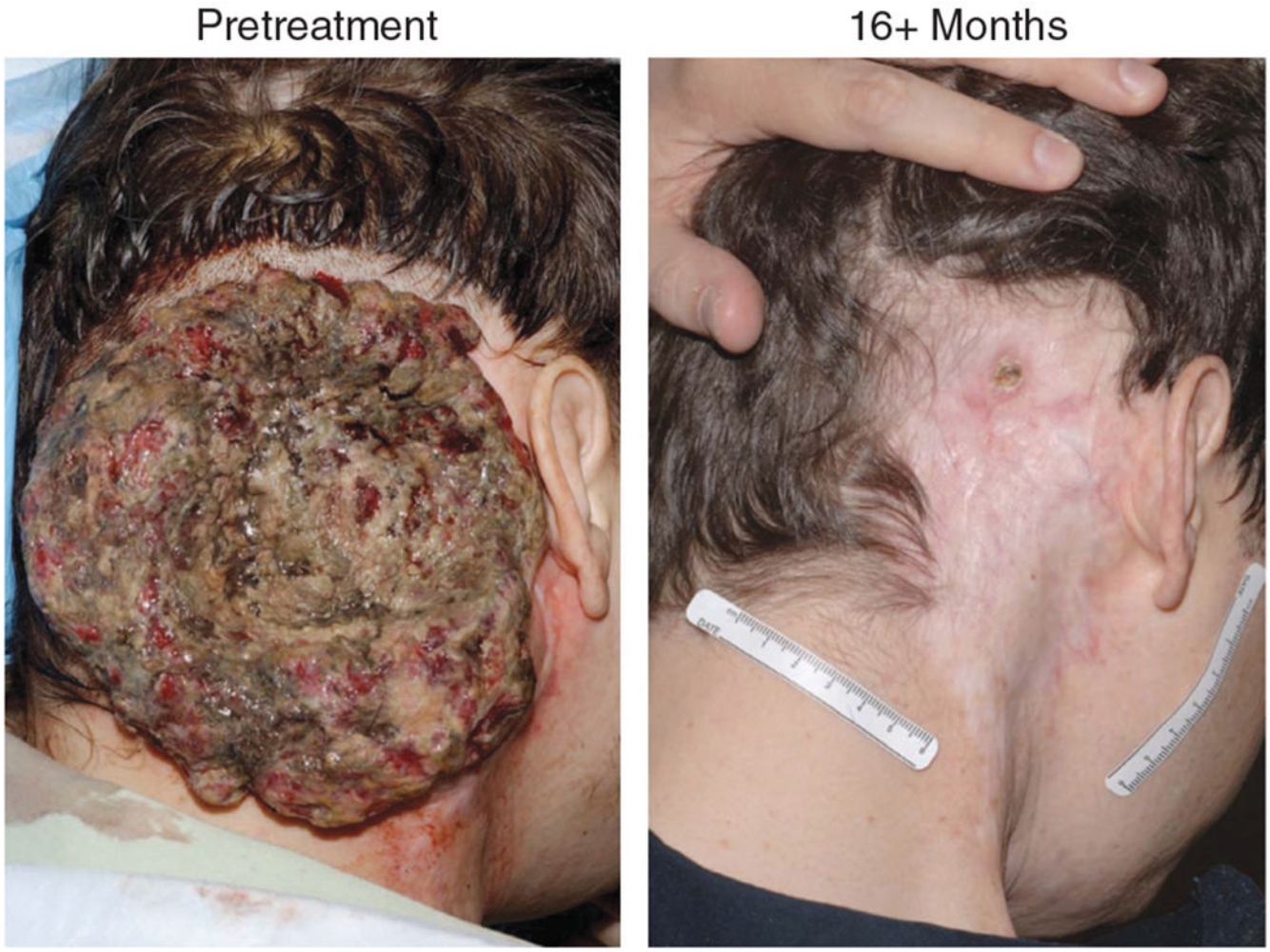

Figure 3. Response of a melanoma tumor to a lymphodepleting chemotherapy regimen combined with adoptive transfer of tumor-infiltrating T cells (Dudley et al., 2002a).
(photo courtesy of Dr Steven A. Rosenberg, Surgery Branch, NCI).

Table 1.

Selected immunotherapy trials for advanced melanoma

	Clinical objective response	References
CTLA-4 blockade		
MDX-010 monotherapy (single dose, 3 mg kg^{-1})	OR 0/7	Hodi et al.(2003)
MDX-010+gp100 peptide vaccination (3 mg kg^{-1} every 3 weeks)	OR 3/14 (CR 2/14; PR 1/14)	Phan et al.(2003)[1]
MDX-010+gp100 peptide vaccination (3 mg kg^{-1} every 3 weeks)	OR 4/29 (CR 2/29; PR 2/29)	Attia et al.(2005)[1]
CP-675,206 monotherapy (0.01–15 mg kg^{-1}, single dose)	OR 4/34 (CR 2/34; PR 2/34)	Ribas et al. (2005)
Dendritic cell-based		
Peptide or tumor lysate	OR 8/32 (CR 2/32; PR 6/32)	Nestle et al. (1998)
Peptide[2] *vs* peptide+GM-CSF[2]	OR 1/13 (CR 0/13; PR 1/13; SD 1/13) OR 2/13 (CR 0/13; PR 2/13; SD 2/13)	Slingluff et al. (2003)[1]
Peptide/lysate *vs* DTIC	OR 3/53 (CR 0/53; PR 2/53; SD 8/53) OR 2/55 (CR 0/55; PR 3/55; SD 10/55)	Schadendorf et al. (2006)[1]
Synthetic peptide vaccine		
g209-2M (gp100-associated antigen)+IL-2, i.v.	OR 13/31 (CR 1/31; PR 12/31)	Rosenberg et al. (1998a)
ACT		
Lymphodepletion	OR 6/13 (CR 0/13; PR 6/13)	Dudley et al. (2002b)
Lymphodepletion	OR 18/35 (CR 3/35; PR 15/35)	Dudley et al. (2005)
Whole-cell vaccine		
Canvaxin® (n=150) *vs* no adjuvant treatment (n=113) (resected AJCC stage IV)	Five-year OS 39% Five-year OS 19%	Hsueh et al. (2002)
Melacine® (n=89) *vs* no adjuvant treatment (resected intermediate-thickness AJCC stage II)	No significant difference in OS and RFS	Sondak et al. (2002)
Melacine+low-dose IFN-α2b *vs* high-dose IFN-α2b (n=604, resected AJCC stage III)	No significant difference in OS and RFS	Mitchell et al. (2007)

CR, complete response; CTLA, cytotoxic T lymphocyte antigen; i.v., intravenous; OR, objective response; OS, overall survival; PR, partial response; RFS, recurrence-free survival; SD, stable disease.

[1]Clinical Response Evaluation according to RECIST.

[2]All patients were also administered low-dose IL-2.