

Immunotherapy of Advanced or Metastatic Melanoma

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Abstract: Melanoma is often evaluated for the development of anticancer immunotherapeutics. Fascinating immune and clinical responses in small numbers of patients have prompted various approaches, ranging from nonspecific immune stimulation to therapies that target specific antigens. Unfortunately, these immune therapies have often shown limited success and objective responses have been seen in only a modest subset of patients. The challenge has been to identify factors that can lead to more consistent clinical benefit and to develop strategies to overcome the obstacles to successful antitumor immunity. Over the last 15 years many immune targets have been identified in cancers and the mechanisms underpinning clinical responses have become better understood. Furthermore, new ways to manipulate anticancer immunity are making it possible to overcome cancer immune evasion and subversion. New therapeutic strategies are resulting from these emerging insights into the relationship between melanoma and the host immune response.

Melanoma as an Immune Target

Melanoma has become the malignancy most commonly investigated with immunotherapeutic approaches in preclinical models and in the clinical setting. A number of early observations suggested that immune recognition might alter the natural history of the disease: regression of primary melanomas, implying an immunologic effect; rarer spontaneous remissions of metastatic disease; and favorable clinical outcomes (regression or prolonged natural history) in association with autoimmune phenomena such as melanoma-associated retinopathy or vitiligo.¹ Tumor-infiltrating lymphocytes have also been associated with a more favorable clinical course.² Nonetheless immune therapies have shown limited success, and objective responses to therapy have been seen in only a modest subset of patients. The challenge has been to identify factors that can lead to more consistent clinical benefit and to develop strategies to overcome the obstacles to successful antitumor immunity. Over the last 15 years, many immune targets have been identified in cancers and the mechanisms underpinning these clinical responses have become better understood. A clearer understanding of the relationship between melanoma and the

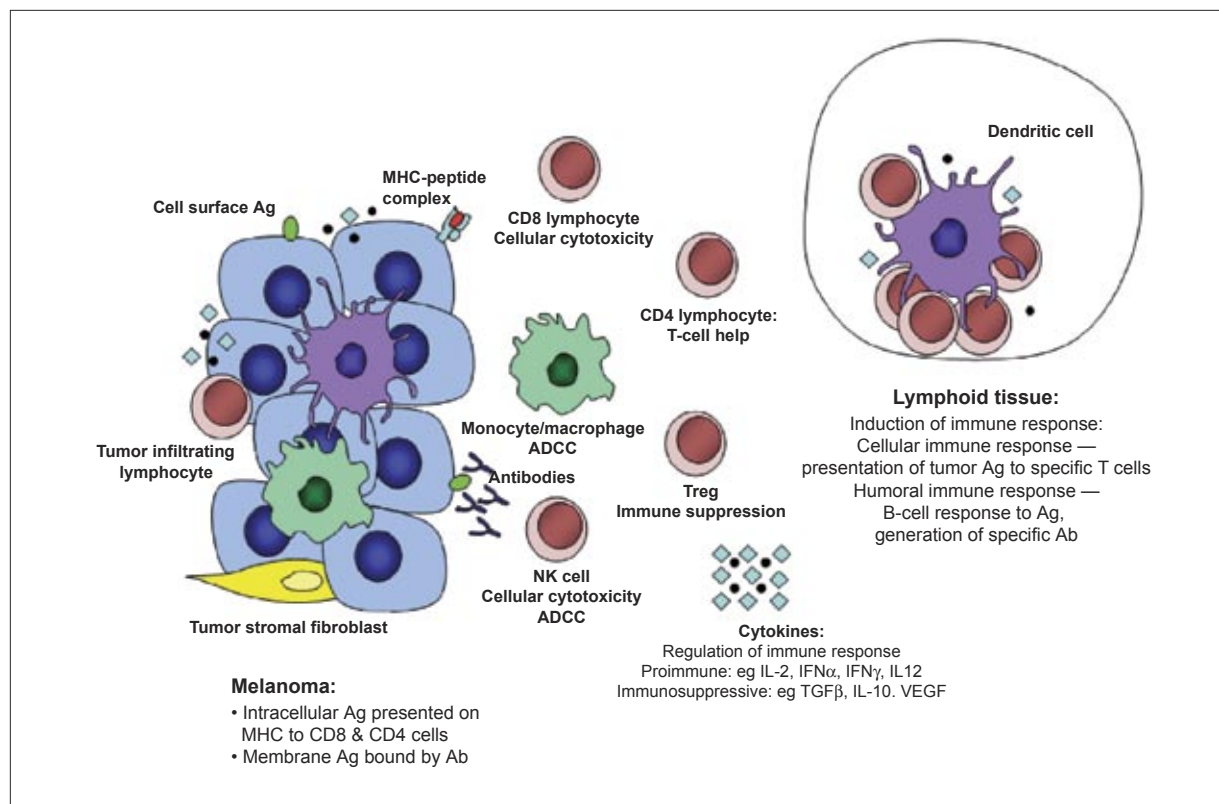


Figure 1. Principal elements involved in anticancer immunity.

host immune response will make it possible to refine therapeutic strategies.

Figure 1 provides a simplified overview of immune effector mechanisms that may play a role in the immune recognition and destruction of melanoma. Specific and nonspecific cellular effectors (CD8⁺ and CD4⁺ T lymphocytes, natural killer [NK] cells) can recognize targets on melanoma, either in the form of peptides complexed with major histocompatibility complex (MHC) molecules (recognized by T lymphocytes) or MHC molecules recognized by NK cell receptors.³ Antibodies against melanoma cell surface antigens may also provide a means of specifically targeting the tumor through mechanisms such as antibody-dependent cellular cytotoxicity or complement-dependent cytotoxicity.⁴ The initiation of these immune responses requires the presentation of tumor antigens. Dendritic cells (DC), macrophages, and even tumor cells themselves can serve as antigen-presenting cells.⁵

Immunosurveillance and Immunoselection

Paradoxically, the intrinsic immunogenicity of melanoma may be an obstacle to effective immunotherapy. Tumor progression can occur in the face of an evolving immune

response due to the selection of variants that either cannot be detected by the immune system (immunoediting)^{6,7} or actively suppress immunity (immunosubversion; Figure 2).⁸ Consequently, the emergence of disease reflects the failure of immune control, the tumor's successful capacity for evading immunity, or both. Table 1 shows a number of mechanisms by which cancers can subvert immunity. As the susceptibility of metastatic melanoma to immunotherapy may be substantially reduced when compared to disease treated in the adjuvant setting, it cannot be assumed that therapies will be equally successful in each setting. Indeed, if an immunotherapeutic strategy is to succeed, the obstacles to immune cancer rejection need to be understood and overcome and different clinical approaches may be required for patients with metastases in contrast to those with minimal residual disease. Patients and their tumors may even warrant "immune profiling" in order to tailor therapy effectively. This review focuses on our emerging understanding of the antimelanoma immune response, insights into the mechanisms that thwart effective immunotherapy in the setting of advanced metastatic disease, and approaches that are showing increasing promise as these obstacles are systematically tackled and overcome.

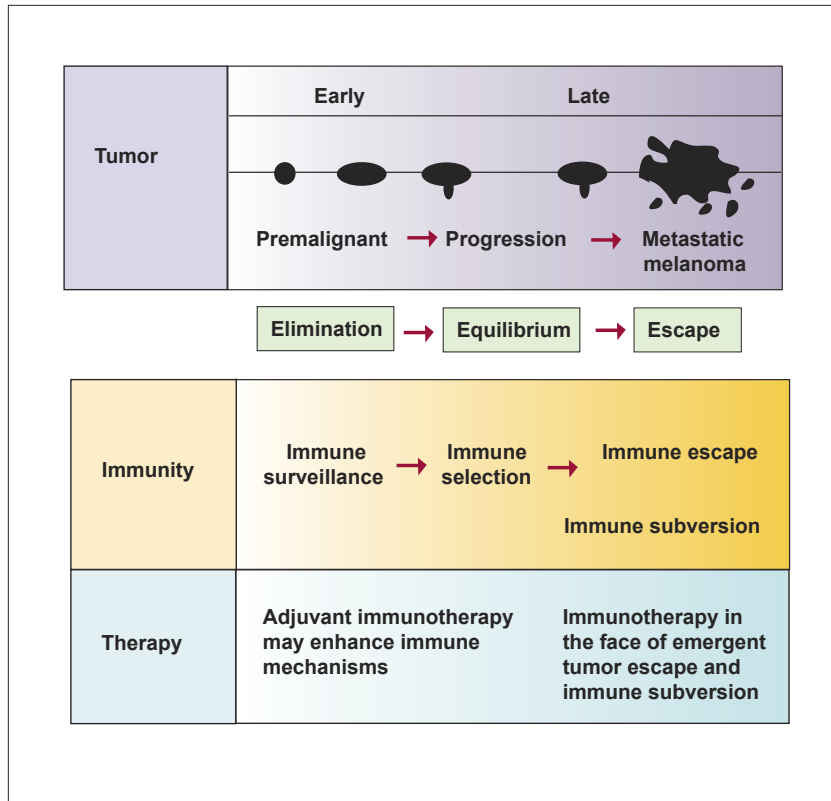


Figure 2. Melanoma progression and immunity.

Past and Current Therapeutic Approaches

A wide variety of clinical immunotherapeutic approaches have been explored in metastatic melanoma with limited clinical benefit. A consistent, small subset of patients has shown clinical regression, but the general failure to raise objective response rates to more than a few percent of patients has resulted in pessimism.⁹

Nonspecific Immunostimulation

This approach is one of the oldest methods used to try to treat cancer and includes some of the most successful outcomes. Treatment of superficial bladder cancer with intravesical instillation of *Bacille Calmette-Guerin* has been proven to reduce the risk of recurrence substantially, although this treatment is not effective against more invasive disease. Since the 1960s cytokines such as interferon (IFN)- α have been known to mediate antitumor effects¹⁰ and have subsequently been used in the treatment of such diverse malignancies as melanoma, renal cell carcinoma, and chronic myeloid leukemia. Interleukin (IL)-2 is also available for clinical use and when given at high doses can cause prolonged remissions in a small proportion of patients with metastatic melanoma or renal cell carcinoma.¹¹ Both IFN- α and IL-2 are associated with

substantial toxicities and are now being supplanted by targeted molecular therapies in these diseases.

The main clinical advantage with IFN- α 2b in melanoma has been seen in selected patients with macroscopically positive lymph nodes but without overt distant metastatic disease. On the strength of initial studies suggesting a survival benefit, high-dose IFN- α 2b was approved for this indication in many countries despite what is often substantial toxicity. Subsequent studies showed that though disease-free survival was increased, no consistent increase in overall survival was seen.¹² As this is the only drug shown to have activity in the adjuvant setting, it is offered to selected patients, although its role remains controversial and use may vary from center to center or be restricted to clinical trials.

Although associated with substantial toxicity, high-dose IL-2 induces response in approximately 16% of patients, with complete remissions in 6%. Approximately 4% of patients overall (ie, two thirds of patients achieving a complete remission) have sustained remission of their metastatic disease and possible cure.¹³ This outcome is qualitatively very different compared with that of patients treated with conventional chemotherapy, in which sustained remissions are very rare in patients with metastatic disease. High-dose IL-2

Table 1. Obstacles to the Immune Recognition and Destruction of Advanced Cancer

	Obstacle	Potential Intervention
Tumor architecture		
	Bulky tumor mass(es)	Debulking: Surgery, radiotherapy, combination therapy
Reduction in antigen presentation		
	Loss of tumor antigen	Upregulation of CT antigens: eg, by using demethylating agents ⁵⁰
	Downregulation or loss of MHC	Upregulation of MHC antigens by IFN- γ ⁷³
	Loss of antigen-processing machinery: TAP-1, LMP2, LMP7, tapasin (described here in colorectal cancer) ⁷⁴	Activation of innate effectors, eg, NK T cells. ⁷⁵
Immunosuppressive tumor microenvironment		
	Molecules elaborated by tumor or stroma: eg, VEGF, IL-6, IL-10, TGF- β , M-CSF, NOS-2, arginase-1, IDO, PGE-2, COX-2, gangliosides ⁷⁶⁻⁷⁸ IDO production depletes tryptophan ⁷⁹	Antagonists: antibodies, soluble receptors, signaling inhibitors (eg, STAT3 inhibition, Arg-NOS inhibitors) ⁶⁸
	Tolerizing antigen-presenting cells impair T cell priming (eg, macrophages, immature DCs, myeloid cells, tumor-infiltrating macrophages ⁷⁰)	Induce myeloid cell maturation (eg, ATRA ⁷⁰) Activation of DC priming: 41BB, anti-CTLA-4 ⁶⁵
	Impaired penetration of effectors	Induce inflammation within tumor micro-environment, eg, with antibody directed against tumor targets such as GD3 ⁸⁰
Resistance to CTL killing		
	<ul style="list-style-type: none"> • Overexpression of P19 blocks granzyme-perforin pathway⁸¹ • Downregulation of death receptors • Reduced caspase 8 activity • Overexpression of FLIP • Overexpressed TRAIL decoy receptors 	
Immunosubversion: active immune suppression		
	Fas ligand production by tumor-killing, tumor-infiltrating lymphocytes ⁸²	
	Loss of CTL cytolytic function and CD4 antitumor activity with tumor progression ^{83,84}	Treat early before loss of T cell function
	Increased Tregs with disease progression ⁵⁸	Anti-Treg strategies: Anti-CD25, denileukin difitox, ⁶³ cyclophosphamide ⁶²

Arg=arginine; ATRA=all-*trans*-retinoic acid; COX-2=cyclooxygenase-2; CT antigens=cancer/testis antigens; CTL=cytotoxic T lymphocyte; CTLA-4=cytotoxic T lymphocyte antigen-4; DC=dendritic cell; FLIP=FLICE-like inhibitory protein; IDO=indoleamine-pyrrole 2,3 dioxygenase; IFN=interferon; IL=interleukin; M-CSF=macrophage colony-stimulating factor; MHC=major histocompatibility complex; NK=natural killer; NOS=nitric oxide synthase; PGE2=prostaglandin E2; STAT3=signal transducer and activator of transcription 3; TGF- β =transforming growth factor- β ; TRAIL=tumor necrosis factor-related apoptosis-inducing ligand; Treg=T regulatory cells; VEGF=vascular endothelial growth factor.

also has the advantage of a short duration of treatment. The mortality rate from high-dose IL-2 has been substantially reduced with recognition of the expected toxicities; however, it should be given only by clinicians experienced in the regimen. Although it proves the concept that an immunotherapeutic approach may have benefit for metastatic melanoma, high-dose IL-2 is clearly not the answer.

Other cytokines that have been evaluated as non-specific immune stimulants include IL-12,¹⁴ granulocyte-macrophage colony-stimulating factor,¹⁵ and Flt3 ligand.¹⁶ Although sporadic reports of clinical benefit have been reported for some of these agents, none has shown consistent evidence of efficacy; for now, their use should be restricted to clinical trials.

Biochemotherapy Some investigators have attempted to combine active nonspecific cytokine-based immunotherapy with more conventional treatments, such as chemotherapy. The rationale is that drug-induced cell death may release tumor antigens that can then be available to induce an immune response, which can then be enhanced by exogenous cytokines. In a series of studies from The University of Texas M. D. Anderson Cancer Center, a regimen of cisplatin, dacarbazine, vinblastine, IFN- α , and IL-2 has been developed and termed "biochemotherapy."¹⁷ Initial single-institution phase II studies suggested that this regimen had high response rates of up to 64%, and many centers including ours adopted this approach for selected patients.¹⁸ Further randomized studies have confirmed an increased response rate in approximately 50% of patients and improved progression-free survival—however with no improvement in overall survival.¹⁹ Alterations in protocols have produced regimens that appear to offer improved response rates with reduced toxicities;²⁰ however, the impact on overall survival remains disappointing. Although it is still being used in many centers, this approach cannot be recommended. It remains possible that a subgroup of patients may benefit, particularly those who have a substantial clinical response early (indicating an effect of chemotherapy) and are thus able to enjoy the long-term benefits that a sustained immune response may bring. This question has been addressed in a trial conducted by the European Organization for Research and Treatment of Cancer, the results of which are awaited.

Adoptive Transfer of Nonspecific Immune Effectors This approach involves administration of cellular agents mimicking those generated by general activation of the immune system. An example is the use of lymphokine-activated killer cells, an approach developed more than 20 years ago.²¹ Responses are infrequent, and the complexities involved in the safe production of

large numbers of cells make the approach unfeasible for most centers. The US National Cancer Institute has also published its experience of depletion of host lymphocytes (immunodepletion) with cyclophosphamide and fludarabine followed by infusion of cultured unpurified lymphocytes and high-dose IL-2.²² This group found that 18 of 35 patients (51%) experienced clinical remission, including three prolonged complete remissions. However, this treatment was toxic and again is not easily applied at most centers.

Specific Immunotherapy

Identification of Specific Antigens The effect of non-specific immunostimulants on melanoma has been both encouraging and disappointing. The demonstration that immune manipulation could induce objective clinical regression established the principle that immune-based therapies could work; however, the relatively low level of activity indicated that such approaches would need to be refined if they were to be helpful for the majority of patients with this disease. It was recognized that anticancer immune responses were likely to be against defined cancer antigens, so their identification held out the promise of developing targeted immunotherapies. Although antigens associated with cancer have been described since the 1950s, it was not until the 1970s that the first human tumor antigens were defined, initially as cell-surface antigens recognized by serum antibodies.²³ More than 10 years later, the first tumor antigens recognized by human T cells were described.^{24,25} Both technologies have been adapted to allow the discovery of new antigens. The SEREX method identifies the anticancer antibody response that arises spontaneously in cancer patients.²⁶ DNA expression libraries (generally constructed from patient tumor samples) are screened using autologous sera to identify those proteins that are recognized by autoantibodies in that patient. (A comprehensive list of antigens identified by SEREX can be found at <http://www.cancerimmunity.org/SEREX/index.htm>.) The definition of T-cell targets is more complex but has also allowed identification of antigens that are possibly more relevant for tumor rejection. Using blood cells from patients with evidence of antitumor responses, it has been possible to isolate T lymphocytes that recognized antigens displayed on autologous tumors.^{25,27} Subsequently, a large number of antigens recognized by T cells have been defined (summarized at <http://www.cancerimmunity.org/peptidedatabase/Tcellepitopes.htm>). Both of these techniques have provided a wealth of targets to investigate. This research has also led to the problem of how to identify which antigens might be the most relevant for clinical use.

Ideally, an immunotherapeutic approach to cancer would be one that leads to regression of established

tumors, induces long-lasting immune responses against an antigen that is specific for cancer, and possibly even protects high-risk patients against the development of cancer. An ideal target antigen should not be expressed on critical normal tissues, but rather be expressed selectively on cancer and be unlikely to be downmodulated in response to immunologic attack. The most commonly selected targets are the melanocyte differentiation antigens Melan-A/MART-1, tyrosinase, and gp100. These are expressed in normal and malignant melanocytes but no other tissues.²⁷ The cancer/testis (CT) antigens are also attractive targets. These are not only expressed in melanoma but also a wide variety of other malignancies. They are not found in normal cells, with the exception of germ cells and placenta.²⁸ Immunologic recognition of these normal tissues is restricted due to their lack of expression of MHC antigens or by an immunosuppressive microenvironment, so CT antigen expression can effectively be considered as restricted to malignant cells. In the case of melanoma, differentiation antigens are expressed in over 90% of cases,²⁹ whereas CT antigen expression ranges from 0% to 70%.³⁰ Expression of the differentiation antigens is usually similar between primary melanoma and more advanced disease; CT antigen expression in melanoma is often acquired as the disease progresses.²⁹ In addition, there is often marked heterogeneity of antigen expression. Without uniform expression of antigens, the selective pressure of an immune response is likely to result in antigen-negative variants emerging as disease progresses, as has been reported for the differentiation antigens.³¹ For the CT antigens, expression can often be limited to relatively few cells in the tumor, which has inevitably raised the question of the benefit of vaccinating against such molecules. For such tumors, vaccines that comprise a mixture of antigens may be necessary. Alternatively, antigens could be selected on the basis of their expression in a critical subset of cells which have the properties of melanoma stem-like cells, discussed below.

Melanoma Vaccines Cancer vaccines have several potential advantages over other immunotherapeutic approaches, including the ability to induce both antibody and cellular responses and to recruit effectors of the innate immune system. Induction of T-cell responses allows the immune system to recognize intracellular protein antigens when appropriate peptides are presented in the context of MHC molecules. Active specific vaccination may also lead to long-lasting antitumor immune memory, which is particularly important if clones of antigen-positive cancer cells persist or if a new cancer or premalignant tissue develops in a field. Many approaches have been used to try to induce active specific immune responses in the setting of cancers, including melanoma. These have included viral tumor lysates, whole tumor cells, peptide eluates, synthetic

peptides, and recombinant proteins.³² A bewildering array of adjuvants and delivery systems have been used, ranging from chemical adjuvants to cellular delivery systems, such as autologous DCs. As tumor antigens became defined, cancer vaccine development increasingly evolved to incorporate approaches that target specific antigens, usually as peptides or proteins. Many of these approaches have been shown to induce immune responses, and there have been tantalizing suggestions of clinical activity, including tumor regressions and long-lasting remissions.²⁷ However, no active specific cancer vaccine has yet been proven to be effective against advanced melanoma.

Antibodies for Targeting of Melanoma Surface Antigens

While many of the vaccination approaches have been designed to elicit cellular immune responses against human leukocyte antigen-restricted targets, cell surface antigens have also been evaluated for immune therapy. A number of clinically useful antibodies are now available for various cancer types such as rituximab (anti-CD20; Rituxan, Genentech/Biogen Idec), trastuzumab (anti-*HER2/neu*; Herceptin, Genentech), cetuximab (anti-epidermal growth factor receptor; Erbitux, Bristol-Myers Squibb/ImClone), and bevacizumab (anti-vascular endothelial growth factor; Avastin, Genentech) used to target B cell lymphomas, breast cancer, and other cancers, respectively. Monoclonal antibodies against melanoma have primarily targeted gangliosides, which are cell-surface glycolipid antigens associated with cells derived from the neural crest.³³ These include the anti-GD3 antibodies R24 (a murine antibody with limited application because it is immunogenic) and, more recently, KM871 (a chimeric antibody reported to have encouraging localization in tumors).³⁴ In some cases these antibodies have been associated with inflammatory responses in tumor deposits but not clinical tumor regressions.³⁵ Other gangliosides might also prove to be suitable targets, and it is possible that the combination of antibodies with other approaches will be useful in the future. Considerable research continues in this field, particularly in the development of new monoclonal antibodies and novel derivatives of these, including antibodies with dual or higher specificity or valency. These constructs will allow targeting of the antibody to tumor cell surface antigens and the recruitment and activation of effector cells, possibly with improved pharmacokinetic and biodistribution properties. However, approaches based on antibodies will always be restricted by their recognition of only cell-surface antigens.

Adoptive Transfer of Antigen-specific T Lymphocytes

This method has been used clinically with reports of good clinical outcomes. One group used autologous blood T cells specific for the melanocyte differentiation antigens Melan-A/MART-1 and gp100.²⁰ Large num-

bers of antigen-specific T cells were cultured in vitro and infused back into patients. Clinical effects including localization of transferred T cells to tumors were observed, particularly with the addition of low-dose IL-2 treatment. Long-lasting engraftment of adopted cells was not observed. The US National Cancer Institute Surgery Branch pioneered the use of tumor-infiltrating lymphocytes for treatment of metastatic melanoma,³⁶ with the rationale that tumor-infiltrating lymphocytes are probably enriched for T cells that have some specificity for antigens expressed in the cancer. In more recent reports, adoptive transfer has been combined with intensive host lymphodepletion, and striking reports of success in suitable patients has been reported.³⁷ To date, however, the complexities and cost of this approach have made it difficult to adopt widely. This group has continued the theme of adoptive passive specific immunotherapy and recently published its experience of a complex approach involving host immunodepletion followed by adoptive transfer of autologous T cells that have been transduced with a retrovirus encoding a specific T-cell receptor.³⁸ A small proportion of patients (2 of 15) showed clinical responses associated with long-lasting engraftment of the transferred cells. This approach has the advantage of being possible without the need for freshly harvested tumor as a source of tumor-infiltrating lymphocytes, as peripheral blood lymphocytes serve as the source of effector cells. Although this advantage would lend it to broader application, these approaches all require substantial resources—currently available at few clinical sites—in order to isolate, transduce, and expand the cells under conditions suitable for human therapy.

Dendritic Cells The potent capacity of DCs to prime T-cell responses and the relative ease of production has made them attractive as cellular vaccines.⁵ They can present tumor antigens from a wide variety of sources, including peptides, proteins, and tumor cell extracts such as lysates, supernatants, exosomes, and RNA. It is unclear whether there are advantages in loading DCs with antigen in the laboratory when vaccines formulated with potent adjuvants can successfully prime immune responses through resident DCs at vaccine sites. In our experience, using a protein vaccine based on the CT antigen NY-ESO-1, there were no clear advantages to the use of DCs compared to protein plus adjuvant, which were administered in a previous trial.^{39,40} Other clinical trials will no doubt determine if this finding is also true using other methods of DC preparation, antigens, and adjuvants.

Overcoming the Obstacles—Emerging Strategies

In order to improve the effectiveness of immune therapy in the metastatic setting, strategies are being developed to

better identify critical targets and facilitate the induction of immune response against them. It will also be essential that regulatory or subversive mechanisms that may suppress these immune responses are overcome.

Refining the Target: Melanoma Stem Cells

There is emerging evidence that a hierarchical structure exists within cancer and that only a minority of cancer cells can form new tumor growths. These have been termed cancer stem cells.⁴¹ In melanoma cell lines, subpopulations enriched for tumorigenicity have been identified by fluorescent dye efflux⁴² and CD20 expression,⁴³ and expression of stem cell markers in melanoma has been demonstrated ex vivo.⁴⁴ More recently, a subpopulation of cells bearing two markers, CD133 and ABCG2, was described in human melanoma samples. Cells bearing these markers were a minority population within the tumors and were tumorigenic, in contrast to the cells that did not display these markers.⁴⁵

Successful immunologic targeting of cancer stem cells is likely to require careful selection of target antigens. Because they play a role in germ-cell biology,²⁸ it has been proposed that the CT antigens may also be expressed by cancer stem cells and may be targets for melanoma stem cells. Although their function is largely unknown, there is increasing evidence that CT antigens may have a functional role in tumorigenesis, perhaps contributing to the stem-like properties of cancer such as self-renewal, proliferation, motility, and differentiation. For example, the CT antigens MAGE-A1 and MAGE-A4 bind a transcriptional regulator that is implicated in the control of cellular differentiation,⁴⁶ and more recently, MAGE proteins have been implicated in mechanisms that protect cells from p53-induced apoptosis.⁴⁷ In an unconfirmed report, CT antigen expression was described in mesenchymal stem cells,⁴⁸ which might raise concerns about toxicity from CT antigen-based vaccines. However none of the vaccine trials targeting these antigens to date has reported autoimmunity to be a problem.⁴⁹

In cases in which melanoma has been largely resected and is present only as minimal residual disease, single cancer cells or small clusters of cells are potentially exposed to circulating immune effectors. Targeting the tumorigenic melanoma stem cells will be critical if metastatic disease is to be prevented,⁴¹ and the CT antigens may prove to be the ideal immune targets in this setting. In contrast, the microenvironment within advanced or metastatic cancer is likely to be immunosuppressive as outlined above, and melanoma stem cells will be protected. In order to effectively eradicate these cells located deep within such a tumor deposit, a two-pronged attack may be warranted: a debulking strategy (whether surgery, radiation, chemotherapy, or immunologically targeting differentiation anti-

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gens) followed by targeting melanoma stem cell–specific antigens to eliminate any residual tumorigenic melanoma stem cells.

Enhancing Antigen Expression

Some antigens, including many CT antigens, are silenced in adult somatic cells due to methylation of the promoter. The use of demethylating agents might allow expression of these antigens in appropriate tissues and immune recognition. 5-Aza-2'-deoxycytidine (decitabine; Dacogen, MGI Pharma) and related molecules have been shown to upregulate CT antigen expression.^{50,51} Furthermore, these agents have proven safe when used for the treatment of myelodysplasia and are currently available for clinical use.⁵² So far, no trials have been reported that combine these demethylating agents with vaccination against defined CT antigens. There is a hypothetical concern that expression of CT antigens in normal tissue may make them targets for autoimmunity, and careful monitoring will be required if this strategy is pursued in the clinic.

Enhancing Cellular Immune Responses: Helper T Cells

Although the major focus of vaccine development has been the induction of a cytotoxic CD8+ T-cell response, it is clear that CD4+ helper T cells also play a critical role in the induction and maintenance of anticancer immunity.⁵³ For peptide vaccines, CD4+ T-cell help can be provided by including peptides that are recognized by CD4+ T lymphocytes. An alternative strategy is to use full-length tumor antigens in the form of proteins or genetic vaccines in the expectation that these will contain antigens recognized by both CD4+ and CD8+ T cells. The tumor antigen NY-ESO-1 is one such antigen, and the majority of patients vaccinated with this protein developed both CD4+ and CD8+ T-cell responses against NY-ESO-1 epitopes.^{39,54} The unusual immunogenicity of this CT antigen may reflect the observation that it is a rich source of MHC class II helper peptide epitopes. It is possible that peripheral immune tolerance does not occur with NY-ESO-1, which is probably a function of its tissue distribution; ie, its expression is restricted to germ cells or malignant tissue and so the immune system remains ignorant rather than tolerant of it. In contrast, the differentiation antigens, which are widely expressed by normal melanocytes, appear to be less immunogenic. It remains to be seen whether their use as full-length antigens combined with appropriate adjuvants can provide the necessary helper responses that will be required to induce long-lasting immunity.

Molecules involved in the costimulation of T-cell responses by DCs include members of the tumor necrosis factor receptor superfamily and their ligands. These include 4-1BB (CD137), OX40 (CD134), and CD27. Engagement of these molecules can enhance the

initiation, expansion, and maintenance of long-lived T-cell responses.⁵⁵ Therapeutic strategies, such as the use of agonistic antibodies that exploit these actions, are being developed as a means of enhancing vaccine responses. Cytokines that regulate lymphocyte proliferation and survival include the IL-2 family members IL-7, IL-15, and IL-21. IL-7 and IL-15 play important roles in the regulation of lymphoid homeostasis.⁵⁶ Though therapeutic administration of these cytokines is one way to potentially enhance cancer immunity, an alternative approach is to increase endogenous levels through lymphodepletion. Lymphotoxic agents, such as high-dose chemotherapy, fludarabine, or even total body irradiation, deplete lymphocytes substantially. In response, a homeostatic increase in serum IL-7 and IL-15 enhances lymphoid reconstitution. When combined with immune therapies, selective expansion of anticancer lymphocytes results. Clinical strategies exploiting this approach have been applied, particularly in conjunction with adoptive T-cell transfer.^{37,57}

Inhibiting Regulatory Mechanisms

Regulatory T Lymphocytes Another potential major barrier to successful antitumor response following vaccination is the inhibition of immunity caused by regulatory T cells (Tregs). These cells control tolerance to self-antigens and play a critical role in suppressing autoimmune responses.⁵⁸ They are characterized by the expression of high levels of the IL-2 receptor β -chain (CD25) and the transcription factor FoxP3. Although they predominantly act to control autoimmunity, they can also suppress antitumor immunity because depletion of these cells enhances antitumor immune responses.^{59,60} It has been proposed that tumor-bearing hosts with advanced cancers have an increased population of Tregs, which might inhibit the tumor-specific T-cell response.⁶¹ Clinical strategies that deplete these cells are therefore potentially capable of enhancing anticancer immunity. A variety of approaches have been taken. These include using cytotoxic chemotherapy such as low doses of cyclophosphamide (300 mg/m²), which enhanced delayed-type hypersensitivity and antibody responses to an early melanoma vaccine.⁶² Other approaches include the use of antibodies against CD25 or a fusion protein, denileukin diftitox (Ontak, Ligand), which is composed of IL-2 and diphtheria toxin. Administration of this agent selectively depleted Tregs without substantial effects on other blood cells. This resulted in enhanced T-cell responses following vaccination.⁶³ Recent studies have suggested another approach to blocking Treg function by targeting Toll-like receptors (TLRs) on DCs. Initially, it was shown that triggering TLR4 or TLR9 on DCs reversed Treg-mediated suppression. Later it was found that TLR8 ligands (eg, oligonucleotides containing a poly-G tail) could inhibit Tregs directly.⁶⁴ Adding TLR ligands to an immunothera-

peutic strategy might therefore boost its effectiveness by helping to overcome Treg-mediated suppression.

Anti-CTLA-4 One of the most interesting recent developments in the immune therapy of advanced melanoma has been the use of antibodies against the immune-suppressive molecule cytotoxic T lymphocyte antigen 4 (CTLA-4). CTLA-4 is mobilized to the surface of activated T cells. Signaling through CTLA-4 dampens the response to specific stimulation through the T-cell receptor and spares the host from the effects of uncontrolled activation of the immune system. Blockade of CTLA-4 can be affected by the use of monoclonal antibodies such as ipilimumab or ticilimumab, and administration of these agents results in dramatic expansion of activated T cells with the potential to mediate antitumor effects as well as autoimmunity. In reported series, up to 20% of patients with metastatic melanoma showed evidence of clinical response, with remissions reported in 10–15% of patients.⁶⁵ Adverse autoimmune events are most frequently seen in responding patients, highlighting the mechanism of action of these antibodies. These include skin rash, inflammatory bowel disease, uveitis, and hepatitis.⁶⁵⁻⁶⁷ These side effects are readily controlled with corticosteroids at doses that do not appear to negate the anticancer effects. Anti-CTLA-4 antibodies are currently being administered in conjunction with vaccines, with cytotoxic chemotherapy, and with cytokines such as IL-2. Randomized clinical trials are now ongoing to determine whether anticancer immunity induced by these agents can be amplified by inhibiting CTLA-4.

Immunosuppressive Tumor Microenvironment The tumor microenvironment is a rich source of molecules with the capacity to subvert immune function. One prominent mechanism is through the accumulation of immature myeloid cells, which can result in local immune suppression.⁶⁸ DCs exposed to these factors can generate dysfunctional and potentially regulatory immune responses. Clinical approaches to counter the effects of myeloid cell-mediated immune suppression include antagonizing the regulatory molecules that lead to these cells accumulating, such as by blocking STAT3⁶⁹ or using agents such as all-*trans*-retinoic acid that differentiate these cells into mature antigen-presenting cells.⁷⁰ In addition, molecular pathways used by these cells can be blocked with agents such as arginine/nitric oxide synthase inhibitors.⁶⁸

Improving Access of T Cells to the Tumor Site Vascular endothelium is a barrier to circulating leukocytes. Penetration of the vascular endothelium can be enhanced in the setting of inflammation.⁷¹ Although the tumor microenvironment may not be inflamed, circulating T cells need

to translocate across endothelium in order to kill tumor cells. This barrier may be overcome by inducing local inflammation, thereby altering the adhesion molecules and chemokines that mediate lymphocyte trafficking. Approaches to enhance access of immune effectors, such as irradiation or intralesional injection of proinflammatory molecules, might be successful only if disease is amenable to local targeting. This approach may be possible for some patients with melanoma, particularly those with in-transit disease localized to a single limb. In order to target widely disseminated disease, monoclonal antibodies provide the most attractive option. As a single agent, the anti-GD3 monoclonal antibody KM871 induced local inflammation in melanoma metastases.⁷² An attractive strategy may be to combine antibodies with approaches that stimulate specific cellular immunity.

Conclusions

It is becoming clear that quite different immunotherapeutic approaches are likely to be required for the immunotherapy of melanoma, depending on whether treatment is in the adjuvant or advanced disease setting. A rich variety of tumor antigens has been identified and work continues to select the most appropriate target for each patient and disease. Whereas early disease may present fewer obstacles to immune recognition and elimination, complex approaches will be required to effectively eliminate advanced metastatic disease. These approaches will almost certainly require the application of combination therapies, designed to bypass or overcome escape mechanisms employed by the tumor. There is tantalizing evidence of efficacy when newer forms of therapy are applied as monotherapies. Combining therapies, perhaps based on an immune profile of the patient and tumor, holds promise of greater success for patients with metastatic melanoma. Many such combination trials are now ongoing in patients with advanced melanoma. A listing of these and other studies currently open to accrual can be found at <http://www.cancer.gov/clinicaltrials/search>.

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standard therapy in this setting. Rituximab maintenance therapy has also been tested in mantle-cell lymphoma, where there was a slight improvement in progression-free survival but not a notable long lasting improvement in overall survival. Trials are examining the best approaches to maintenance therapy in other types of lymphoma; for example, whether therapy should be continued until relapse or for a set period of time, and what doses and schedules are ideal.

H&O What further controversies remain with rituximab maintenance therapy?

JV There is controversy regarding the increased risk of infection due to neutropenia in patients who receive rituximab for a long period of time. Certain rare infections have been observed in patients receiving rituximab, such as reactivation of hepatitis B or progressive multifocal leukoencephalopathy. Another controversy

is whether maintenance therapy leads to increased resistance to rituximab. All patients who receive rituximab appear to develop resistance eventually, but the concern is whether maintenance therapy causes patients to develop this resistance sooner.

Suggested Readings

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