Update on Immunotherapy for Melanoma

Antoni Ribas, MD, PhD, Los Angeles, California

Abstract
Several experimental immunotherapy approaches and standard therapy with high doses of interleukin (IL)-2 can cause prolonged objective responses in some patients with metastatic melanoma. Experimental immunotherapy approaches in clinical development include 1) cytokines such as IL-7 and IL-21, 2) cytokine–antibody fusion proteins or immunocytokines, 3) whole tumor cell vaccines, 4) genetically modified tumor cells, 5) heat shock protein vaccines, 6) peptide vaccines, 7) dendritic cells pulsed with tumor antigens, 8) tumor antigen-naked DNA vectors, 9) recombinant viral vectors (either alone or in a prime boost schedule), 10) adoptive transfer of cloned tumor antigen-specific T cells, 11) Toll-like receptor ligands, 12) antagonistic antibodies to the cytotoxic T-lymphocyte antigen 4 (CTLA4, CD152), and 13) activating antibodies to CD40 and CD137 (41-BB). These improved approaches to induce cytotoxic T-cell responses to tumors are based on a more detailed understanding of the immune system activation and regulation. The higher response rates with modern immunotherapy approaches may allow exploration of the molecular mechanisms that make tumor targets sensitive or resistant to immunotherapy. (JNCCN 2006;4:687–694)

Knowledge of how the immune system recognizes and attempts to control cancer growth and development has improved remarkably.1-4 Researchers now know that the immune system has a pivotal role in controlling emerging premalignant lesions that develop into invasive tumors.1 Mice with immune deficiencies develop carcinogen-induced tumors at increased frequency, and human subjects with inherited or acquired immune deficiency states have a higher incidence of cancers.1 In addition, large series have shown that primary tumor infiltration by lymphocytes is a good prognostic factor for melanoma, ovarian carcinoma, and colon carcinoma.2-3

For several reasons, melanoma is among the tumors for which immunotherapy has been most actively tested and accepted mode of therapy.2,3,10 Documentation shows that the lesions in a small subset of patients with primary skin melanoma undergo spontaneous remission, and some spontaneous remissions have been seen even in patients with advanced melanoma.2 The notorious resistance of melanoma cells to standard cytotoxic therapies, such as chemotherapy and radiation therapy, has enabled ample testing of other modes of therapy. Sampling and study of lymphocytes infiltrating tumors is facilitated by their relatively high percentage compared with other cancers. As a result, researchers now have a fair understanding of the antigenic restriction of tumor infiltrating lymphocytes (TILs) in melanoma and how they are regulated.2,9,10 However, most attempts to develop cancer immunotherapy strategies have failed in the past several decades.11 New immunotherapy approaches are based on failed clinical experiences and novel understanding of the immunobiology of antitumor responses (Table 1).

The extremely long, objective tumor regressions experienced by a small subset of patients are a hallmark of effective melanoma immunotherapy. Mixed responses and periods of disease stabilization have been quoted by some investigators as evidence of antitumor activity to highlight the benefits of strategies that otherwise have marginal antitumor activity. However, without objective remission criteria from the World Health Organization (WHO), Response Evaluation Criteria in Solid Tumors (RECIST), or a control arm for comparison, these observations of mixed responses and disease stabilization are of little significance in melanoma.11
Immunotherapy Approaches for Melanoma Aimed at Stimulating Cellular Responses to Cancer

Cytokines

Interleukin (IL)-2 (aldesleukin, Proleukin, Chiron, Emeryville, CA) is approved by the U.S. Food and Drug Administration (FDA) for treating metastatic melanoma. IL-2 has no direct cytotoxic effects on melanoma and requires immune system cells for its antitumor effects. The licensing of this immunotherapy was based on a subset of patients with long-term survival in phase II trials, but randomized trials have not been conducted to show that IL-2 improves overall survival. Several major drawbacks have limited the routine use of this immunotherapy in patients with melanoma. Because IL-2 is a highly toxic regimen requiring inpatient treatment in a setting similar to an intensive care unit and administration by highly trained staff, its clinical use is limited to mostly large academic centers. More importantly, most patients experience expected grade 3 and 4 toxicities and no benefit, because fewer than 10% experience objective response and long-term remission.

Interferon (IFN)-α2b (Intron-A, Schering Corporation, Kenilworth, NJ) is approved in the United States for the adjuvant treatment of high-risk melanoma based on 3 large randomized trials, which showed a small but reproducible favorable impact on progression-free survival and questionable impact on overall survival. The development of autoantibodies after administration of IFN-α2b seems to be associated with lower relapse rates. IFN-α2b and peginterferon alpha also have shown some evidence of antitumor activity in advanced melanoma.

Granulocyte-macrophage colony-stimulating factor (GM-CSF or sargramostim, Leukine, Berlex, Inc., Montville, NJ) has been tested in the adjuvant treatment of resected stage IV melanoma in a phase II study. This study was conducted in the setting of no evidence of disease after complete surgical resection of melanoma lesions, and patient outcome was compared with historical controls. Therefore, with the lack of a concomitant control arm, the value of GM-CSF as adjuvant treatment for surgically resected melanoma is difficult to assess. An adequate randomized trial is underway. Other cytokines in clinical development in melanoma include IL-7, IL-12, IL-18, and IL-21, all of which have recognized ability to activate cytotoxic T cells.

Biochemotherapy

Treatment involving chemotherapy agents combined with cytokines such as IL-2 or IFN-α2b has been called biochemotherapy. These combinations were intensely researched in the past decade, and were once considered standard treatment options by some melanoma specialists. Phase II studies reported response rates beyond those observed with chemotherapy agents alone, although with markedly worse toxicities. Most data from 9 randomized trials testing biochemotherapy regimens confirmed that patients with metastatic melanoma responded better to biochemotherapy than to chemotherapy or cytokines alone, although most responses were of short duration. None of these trials showed an increase in overall survival compared with chemotherapy or cytokine therapy alone. One reason for this may be that these combinations potentiate the cytotoxic activity of chemotherapy but negate the benefit of long-term disease control seen in patients experiencing immune-mediated tumor regressions alone. Cytokine-activated lymphocytes may be more sensitive to the cytotoxic effects of chemotherapy than most melanoma cells. Because of this extensive clinical testing and lack of survival advantage,

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Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin.
further testing of chemotherapy as currently designed may not be warranted.\textsuperscript{20}

**Immunocytokines**

The development of cytokines for cancer therapy has been limited by the need to administer high doses of the cytokines for prolonged periods to achieve the minimally required active biologic concentrations at the tumor cell–immune cell interaction where they are needed. Immunocytokines were developed to deliver the cytokines to the tumor sites without systemic toxicities.\textsuperscript{22} These are antibodies targeted to melanoma cell surface molecules, which are chemically or genetically linked to immune-stimulating cytokines. Gangliosides have been used as surface receptors to target melanoma antibodies, but they are not tumor-specific surface molecules.\textsuperscript{21,22}

**Whole Tumor Cell Vaccines**

Multiple formulations of cancer vaccines were developed based on the administration of lysates or inactivated whole tumor cells, and have been extensively studied over the past 40 years. The rationale is that antigenic epitopes from tumor cells may be taken up by host antigen-presenting cells (APCs), resulting in the activation of antitumor T cells. Several large randomized trials used these vaccines in patients with metastatic melanoma or as adjuvant treatment in those with resected melanoma.\textsuperscript{23} Recently completed randomized trials of the allogeneic tumor cell vaccines Melacine (Corixa, Seattle, WA) and Canvaxin (CancerVax, Carlsbad, CA) have shown that overall these vaccines have little to no patient benefit in terms of survival advantage.\textsuperscript{24} Most experts now recognize that the complex regulation of the immune system and the immune suppressive factors produced by tumor cells make it unlikely that these tumor lysates or inactivated tumor cells, even together with strong immunologic adjuvants, will overcome peripheral tolerance to tumor antigens.\textsuperscript{25-27}

**Genetically Modified Tumor Cell Vaccines**

Gene transfer techniques into cancer cells were developed in the late 1980s based on the hypothesis that insertion of immune stimulatory genes into cancer cells may allow the production of antitumor vaccines.\textsuperscript{28} Side-by-side testing of different immune stimulatory genes inserted into cancer cells provided evidence that those expressing GM-CSF, with its ability to attract host APCs, were the most powerful in animal models.\textsuperscript{29} Translating this approach to humans required proliferating cancer cell lines from patients’ tumors to be established ex vivo and effectively inserting the cytokine genes. Manufacturing these autologous tumor vaccines outside of pilot trials was difficult, and the vaccines had a low ability to induce clinically relevant responses.\textsuperscript{28-31} To directly recapitulate the procedures used in animal models, a minimum of several weeks to months is needed to manufacture autologous genetically-modified tumor vaccines, during which a significant number of patients may experience tumor progression and become ineligible to receive the genetically-modified tumor cells.\textsuperscript{32} To improve the manufacturing process and generate vaccines amenable for wider human testing, researchers introduced variations. These include genetic manipulations of established tumor cell lines hypothesized to provide shared antigens, and genetic modifications of normal skin fibroblasts readministered together with short-term cultures of autologous tumor cells.\textsuperscript{32}

**Ganglioside Vaccines**

Gangliosides are carbohydrates aberrantly expressed on the surface of melanoma cells. When reformulated with immunologic antigens, they can induce antibody responses that are cytolytic in vitro against melanoma cell lines.\textsuperscript{33} Vaccination with gangliosides has been tested in several clinical trials for melanoma. The largest was a phase III trial comparing a GM2-KLH ganglioside vaccine (GMK; Progenics, Tarrytown, NY) with high-dose IFN-\textalpha-2b in the adjuvant treatment of high-risk melanoma.\textsuperscript{34} This trial was stopped early because IFN-\textalpha-2b showed a clear benefit compared with the ganglioside vaccine. Another large randomized trial testing this ganglioside vaccine has been conducted in Europe, but study results are not yet available.\textsuperscript{35} Other gangliosides in different formulations are being developed as candidate melanoma vaccines, such as GM1, GM3, GD2, and GD3.\textsuperscript{33,36,37}

**Heat Shock Proteins**

Tumor vaccine development requires tumor antigens to be purified and separated from their poorly immunogenic (arguably tolerogenic) native form in the tumor cells. Heat shock proteins, which are proteins linked to cellular stress molecules, were seen to function as chaperones for tumor antigens, because they bind peptide antigens.\textsuperscript{38,39} Heat shock proteins can be purified from tumor lysates and administered to patients. A randomized melanoma trial failed to reach the primary end point of overall survival. However, a retrospective analysis showed that patients with M1a
These subset analysis results require further validation in a prospective clinical trial.

**Peptide Vaccines**

Tumor antigens recognized by cytotoxic T cells are 8 to 11 amino acid-long peptides derived from intracellular proteins presented on the cell surface by major histocompatibility complex (MHC) class I molecules. These peptides can be synthesized and administered with immunologic adjuvants as vaccines. In humans, the MHC class I complex, termed human leukocyte antigens (HLA), comprise widely polymorphic proteins inherited according to Mendelian rules. Among the more than 60 HLA subtypes, some are more common, most notably HLA-A*0201 (HLA-A2.1 in older terminology). Each subtype has different binding rules for peptide epitopes, and peptides presented by one may not bind to another. Therefore, only matched HLA subtypes can present synthetic tumor-derived peptides when used as vaccines. Their ease of synthesis has allowed them to be widely tested in clinical trials. Studies showed that a prolonged period of repeated immunizations is required to first detect peptide-specific T cells, which is a limitation for patients with progressive metastatic melanoma. One study reported a high rate of tumor responses when the vaccine was coadministered with high-dose IL-2, but whether this was caused by IL-2 alone or the combination was unclear. When these peptide vaccines were tested in the adjuvant setting, where time allows multiple immunizations, they did not clearly prevent tumor relapse. Overall, peptide vaccination produces very few objective responses, and may not be a valid therapeutic option using current approaches.

**Dendritic Cell Vaccines**

The APCs with greatest ability to activate naïve T cells are the dendritic cells. These cells can be differentiated ex vivo from monocyte or hematopoietic stem cell precursors using cytokine-containing cell cultures. They can then be loaded with tumor antigens and readministered as vaccines. Tumor antigen loading in clinical trials has occurred more frequently in the form of peptides, requiring HLA matching. If autologous dendritic cells are loaded with tumor lysates, immunization relies on the endogenous processing of peptide epitopes, and therefore does not require HLA matching.

Another approach has been to genetically engineer dendritic cells to express tumor antigens or immune stimulatory molecules. Overall, dendritic cell vaccines have shown the ability to expand tumor antigen-specific T cells, but the limited ability to induce tumor regressions. In one randomized trial, tumor antigen-loaded dendritic cell vaccines had the same response rate as dacarbazine in patients with metastatic melanoma.

**Plasmid DNA, Recombinant Viral Vector, and Prime-Boost Immunization**

The cloning of tumor antigen genes opened the door to their administration as tumor vaccines. Certain cells, such as fibroblasts and myocytes, can take up plasmid DNA administered by simple needle injection and express transgenes. This approach showed that immunization with tumor antigens had the ability to prime T cells against xenoantigens but little ability to break self-tolerance.

Xenoinmunization, whereby a tumor antigen gene sequence from one species is used to immunize subjects of another species (e.g., a melanoma antigen gene from humans used to immunize mice), has been shown to efficiently prime tumor antigen–specific T cells. Immunization with recombinant viral vectors results in higher levels of transgene expression at the expense of higher immunogenicity of the vector, which may limit or skew the immune response to the tumor antigens, has been shown to expand tumor antigen-specific T cells.

These approaches can be combined sequentially in what have been called prime-boost strategies. Original reports were based on priming a T-cell response to the tumor antigen transgene using naked DNA immunization followed by a viral vector boost expressing the same tumor antigen. The term prime-boost is now generally used for any combination of priming and boosting, whether a naked DNA plasmid, a viral vector, or the whole protein is administered.

**Modulation of Costimulatory Signaling**

Two signals from APCs are required to activate T cells: the antigen presented by MHC molecules and costimulation with the B7 family of molecules. The immune system seems to have developed multiple safety mechanisms to avoid overactivation and responses to self. A major mechanism is the induction of a negative costimulatory receptor on activated T cells called
the cytotoxic T-lymphocyte-associated antigen 4 (CTLA4 or CD152). Blockade of this negative signaling using specific antibodies led to tumor regression in mice. In patients with melanoma, CTLA4 antagonistic antibodies broke self-tissue tolerance, which resulted in autoimmune phenomena and also objective and durable tumor regressions in some patients.

Other negative immune signaling pathways are potentially amenable to pharmacologic blockade. These include the negative costimulatory receptors programmed death-1 (PD-1) and B and T lymphocyte attenuator (BTLA), secreted molecules such as transforming growth factor β (TGF-β), and immune down-modulating enzymes such as cyclooxygenase-2 (COX-2) and indoleamine 2,3-deoxygenase (IDO).

Activating antibodies that target costimulatory molecules are being clinically developed. The tumor necrosis factor super-family receptor CD137 (4-1BB) provides costimulatory signals to T cells. Administering activating antibodies to CD137 caused tumor regression in animal models, which prompted the clinical testing of an antibody (BMS-663513) currently under development for melanoma. An activating antibody to CD40 (CP-870,893) is also being developed that has shown single-agent activity in patients with metastatic melanoma in phase I trials (Robert H. Vonderheide, unpublished data, 2006). CD40 is required for generating fully functional CD8+ cytotoxic T lymphocytes because it bypasses the need for CD4+ T-helper cells.

Adoptive Transfer Immunotherapy

The goal of cellular immunotherapy is to expand tumor antigen-specific T cells with cytolytic activity against cancer cells. Purification, ex vivo activation and expansion, and readministration of antitumor cytotoxic T cells may bypass the limitations of active immunization with vaccines. Early strategies used in clinical trials, such as administering TILs and lymphocyte-activated killer cells (LAKs), were limited by their relative inability to home back to tumors. Subsequent approaches using cloned tumor antigen-specific T cells generated through specific ex vivo expansion using antigenic stimulation were limited by the short survival of the adoptively transferred T cells. A major breakthrough occurred when experts exploited the process of homeostatic proliferation of T cells when infused into lymphopenic hosts, which provided a selective advantage for the adoptively transferred T cells to proliferate and repopulate the host. Using chemotherapy conditioning followed by the adoptive transfer of cloned tumor antigen-specific T cells and high doses of IL-2 (as a source of helper cytokine to sustain T-cell proliferation), the highest reported rates of responses in metastatic melanoma to any therapeutic intervention have been achieved. Despite its success, this approach is unlikely to be widely used in patients with metastatic melanoma. Tumor antigen-specific T cells only can be cloned in a few patients; the process is lengthy, costs several tens of thousands dollars per patient, and requires highly trained personnel; and the tumor antigen-specific cloned T cells are stimulated with synthetic peptides, which pose HLA restrictions. Cloned tumor antigen-specific T cells can be generated more rapidly by genetically engineering them with the genes for the receptors that provide T-cell restriction (the T-cell receptor).

Toll Receptor Ligands

The immune system rapidly reacts against many pathogens by recognizing shared patterns among them that are not present in mammalian cells. These pathogen-associated molecular patterns bind to specific pattern-recognition receptors similar to the Toll receptors in Drosophila. Ten members of the Toll-like receptor (TLR) family are now recognized in humans. TLR triggering causes powerful immune-activating signals. For example, unmethylated deoxyinosine-deoxyguanosine (CpG) motifs are frequent in the prokaryotic genome of bacteria but infrequent in eukaryotic genome, and are recognized by TLR 9. Several purified or synthetic forms of TLR ligands are in clinical development and being tested in patients with melanoma (Table 1).

What Limits Effective Immunotherapy?

A common ceiling of antitumor activity is apparent in melanoma immunotherapy. In the best-case scenarios, only 5% to 15% of patients experience objective tumor responses with active immunotherapy strategies. Much effort has been put into developing more powerful vaccines that have an enhanced ability to stimulate and expand tumor antigen-specific T cells, but objective tumor responses continue in the same range. The additional modulation of negative immune signaling may produce somewhat improved responses, but published results are similar to those for tumor vaccines. Adoptive transfer immunotherapy bypasses the step of APC activation of
T cells and has led to higher response rates when combined with myeloablative and IL-2. However, some patients still have progressive tumors in the presence of extremely large numbers of circulating tumor antigen-specific T cells, indicating the intrinsic limitations of immunotherapy activity. These limitations could be a result of low levels of antigen ligands for T cells, dominant immune-suppressive milieu within tumors, or lack of cancer cell sensitivity to the proapoptotic signals delivered by activated immune system effector cells.

Who Should Undergo Immunotherapy?

Early clinical trials conducted by a limited number of investigators in large academic centers often show higher response rates to immunotherapy than larger multi-institutional clinical trials. One main reason may be a “cherry-picking” effect, with a strong selection bias in early studies. Investigators testing novel immunotherapy strategies may tend to select patients with suspected slower tumor growth rates, or may have patients referred to them for clinical trial participation who have clinical characteristics that suggest the immune system is acting on the natural course of their tumors. Examples include: in-transit metastasis; metastasis limited to the skin, lymph nodes, and lung; evidence of codeveloping autoimmune diseases, such as vitiligo or thyroiditis; long intervals between the primary tumor and the development of systemic metastasis; and oligometastatic lesions with slow tumor growth. On the contrary, metastasis in visceral sites other than the lung, high levels of serum lactate dehydrogenase, rapid tumor kinetics, or simultaneous development of multiple metastases in the immediate preceding period are indicators that patients are less likely to respond to immunotherapy. When the same immunotherapy approaches are tested in multicenter clinical trials, which may have the goal of entering a larger number of patients rather than selecting those with clinical characteristics that suggest potential “immune-sensitivity,” the response rates invariably drop. Therefore, perhaps not all patients are candidates for immunotherapy. However, whether patients will respond to immunotherapy is currently unpredictable, and therefore the immunotherapy field has the major challenge of defining signatures of tumor response or resistance at the molecular level, enabling scientific definitions of “immune-sensitivity” and “immune-resistance.”

Conclusion

Clearly, a small subset of patients with widely metastatic melanoma does respond to various immune-activating strategies, and these responses last years in most cases. Although not all metastatic melanomas may be treated through modulating the immune system, improved knowledge of how to activate, modulate, and target antitumor T-cell responses is resulting in new generations of immunotherapy approaches with consistent response rates. Improved knowledge of melanoma biology may allow molecular patterns to be defined that result in response or resistance to immunotherapy, which may allow interventions to be explored that have the potential to pharmacologically overcome resistance to immunotherapy.

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