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EDITORIAL

# Getting Melanoma Cells to Stimulate With Frequency

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Strategies for the delivery of antigenic peptides to patients with advanced malignant melanoma include a variety of novel approaches, such as the administration of recombinant DNA-encoding antigenic-peptide sequences; mRNA transcripts and peptides collected directly from patient tumors; recombinant replication-deficient adenoviral and retroviral vectors encoding tumor antigens; synthetic peptides modified for enhanced binding to antigen-presenting cell (APC) and human leukocyte antigen (HLA) molecules; allogeneic tumor cell lysates; peptide-loaded dendritic cells; tumor cell-dendritic cell fusion products; and irradiated autologous tumor cell preparations.[1] Thus far, it is not clear that any one method is superior for the generation of antigen-specific T cells, and the induction of durable clinical responses has remained an elusive goal. It may develop that multiple methods of vaccination will be employed depending upon the specific disease setting or the type of antigen that is being delivered. One area of research that may enhance the success of vaccination strategies is the development of improved vaccine adjuvants.

The development of an effective immune response and long-lasting immunologic memory in response to the delivery of an antigenic peptide requires the generation of a pool of antigen-specific T cells. Coadministration of an adjuvant with the vaccine can enhance the activity of weak immunogens. Vaccine adjuvants may function in several ways. An adjuvant may act as a depot for the vaccine, thus prolonging the time period over which an antigen is presented to APCs. Alternatively, an adjuvant may be employed as a means to change the character, number, or activation state of APCs at the vaccination site. Finally, an adjuvant may be used to alter the immunologic pathway by which the protein is being presented or processed, thus steering the immune system toward either a cell-based or an antibody-based response to the antigen. Cytokines are of particular interest to this field because they affect which arm of the immune system is stimulated to respond to the antigenic stimulus. Interleukin-12, interleukin-2 (IL-2), and interferon-$\alpha$ are examples of cytokines that have been tested as adjuvants in the setting of metastatic malignant melanoma.[2-4] However, laboratory and clinical studies indicate that granulocyte-macrophage colony-stimulating factor (GM-CSF) may have particular advantages as a vaccine adjuvant.

GM-CSF stimulates the activation, maturation, and migration of dendritic cells (DCs) and macrophages and induces their expression of class II major histocompatibility complex molecules.[5] Using a murine model, Disis et al[6] showed that GM-CSF was comparable to complete Freund's adjuvant in augmenting the immune response to both tetanus toxoid and a peptide sequence derived from the rat *neu* protein (homologue of human c-erb-B2). Importantly, peptides administered without GM-CSF were essentially nonimmunogenic. Dranoff et al[7] used a murine melanoma model to evaluate seven different cytokines for their ability to enhance the immunogenicity of irradiated B16 tumor cells, which alone do not stimulate significant antitumor immunity. Tumor cells transduced with a retroviral construct for GM-CSF exerted potent, long-lasting, and specific antitumor immunity that required both $CD4^+$ and $CD8^+$ T cells. Tumor cells expressing other cytokines were either poorly immunogenic (IL-2, IL-5, interferon-$\gamma$ [IFN-$\gamma$], tumor necrosis factor-$\alpha$ [TNF-$\alpha$]), or weakly immunogenic (IL-4 and IL-6). Vaccination of stage IV melanoma patients with irradiated, autologous melanoma cells engineered to secrete GM-CSF by adenovirus gene transfer led to an antigen-specific T-cell response (as measured by a delayed-type hypersensitivity reaction to nontransduced tumor cells) and measurable lymphocytic infiltration of tumor deposits in a significant proportion of patients, as well as one complete response.[8] Likewise, a multipeptide melanoma vaccine elicited superior T-cell immunity when injected as an emulsion with GM-CSF as compared



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with being administered via peptide-pulsed, monocyte-derived DCs.[9]

In this issue, Luiten et al[10] report the results of a vaccine trial in which patients with stage IV malignant melanoma were injected with irradiated autologous tumor cells that had been transduced retrovirally with the *GM-CSF* gene. Patients were randomly assigned to receive three injections of either $5 \times 10^6$ or $5 \times 10^7$ transduced tumor cells at 3-week intervals. The authors hypothesized that local production of GM-CSF by injected tumor cells would promote an inflammatory milieu as well as promote the migration and maturation of DCs, thereby enhancing the immunogenicity of the injected cells. Vaccine production was successful in 56 (88%) of 64 patients, and production of GM-CSF by transduced tumor cells was verified in each instance; because of disease progression, only 28 patients completed all three planned vaccinations. In general, the vaccinations were well tolerated. However, pruritus at the vaccination site was observed with 85% of the vaccinations, and 30% of patients experienced fever or headache. Systemic levels of GM-CSF were not detected after vaccination.

There were no objective clinical responses in the 28 vaccinated patients; however, long-term survival of more than 60 months was observed in six patients who had been rendered free of disease as a result of the surgery for tumor acquisition or prior radiotherapy. The site of metastatic disease in all six of these patients was in the subcutaneous tissues or in lymph nodes, and only three had received prior systemic therapy for their disease. These patients were distributed equally between the two vaccine dose levels. It is important to note that two of the long-term survivors exhibited an early recurrence at 2 to 6 months, underwent complete resection of their metastasis, and then experienced a long period of stable disease. These characteristics, along with the fact that this trial design selected against the participation of patients with aggressive disease (the median time from the excision of tumor to the start of vaccination was 10 weeks), raise the possibility that the favorable outcome experienced by these six patients may have been a result of the presence of tumors with a favorable growth profile or the administration of the vaccine in the setting of minimal residual disease. By way of comparison, other investigators[11] have reported a 39% 5-year overall survival rate for melanoma patients who received a therapeutic cancer vaccine after complete resection of stage IV disease.

Evidence that the vaccine was able to stimulate an antigen-specific T-cell response comes from several sources. Two patients with prolonged survival developed vitiligo lesions at multiple sites several months after vaccine administration, indicating that specific immunity against melanocyte antigens had been induced. Indeed, analysis of peripheral T cells and a skin biopsy revealed the presence of T cells reactive to MART-1 peptide bound to HLA-A2 in one of the vitiligo patients. Immunohistochemical analysis of tumors resected postvaccination revealed greater T-cell infiltration of tumor tissue in the patients who received the high-dose vaccine as compared with those in the low-dose group. Also, nontransduced tumor cells induced delayed-type hypersensitivity reactions in all patients after vaccination. An extensive panel of HLA/peptide tetramers was developed and used to monitor the effects of the vaccine regimen on T-cell immunity in 25 of the 28 patients who completed the regimen. Eight of these patients were HLA-$A2^+$. Melanoma-reactive T cells increased in three patients who received the high-dose vaccine, one of whom was a long-term survivor who developed vitiligo. However, the remaining patients showed low or undetectable levels of melanoma-specific T cells that did not increase with vaccination.

The in-depth immunologic correlative studies performed in this study support the authors' contention that the vaccine regimen was capable of inducing antigen-specific T cells with antitumor effects. However, the lack of objective responses and the resection of metastases during the course of follow-up suggest that the vaccine may be most effective in the setting of minimal tumor burden. The present study provides further support for the use of GM-CSF as a vaccine adjuvant and demonstrates the utility of intensive immunologic monitoring of vaccine patients. Additional refinements, as well as larger randomized studies will be necessary to determine the true value of this treatment in the setting of malignant melanoma.

### *Author's Disclosures of Potential Conflicts of Interest*

The author indicated no potential conflicts of interest.

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## REFERENCES


1. Yannelli JR, Wroblewski JM: On the road to a tumor cell vaccine: 20 years of cellular immunotherapy. Vaccine 23:97-113, 2004
2. Lee P, Wang F, Kuniyoshi J, et al: Effects of interleukin-12 on the immune response to a multipeptide vaccine for resected metastatic melanoma. J Clin Oncol 19:3836-3847, 2001
3. Slingluff CL Jr, Petroni GR, Yamshchikov GV, et al: Immunologic and clinical outcomes of vaccination with a multiepitope melanoma peptide vaccine plus low-dose interleukin-2 administered either concurrently or on a delayed schedule. J Clin Oncol 22:4474-4485, 2004
4. Vaishampayan U, Abrams J, Darrah D, et al: Active immunotherapy of metastatic melanoma with allogeneic melanoma lysates and interferon alpha. Clin Cancer Res 8:3696-3701, 2002
5. Chang DZ, Lomazow W, Joy Somberg C, et al: Granulocyte-macrophage colony stimulating factor: An adjuvant for cancer vaccines. Hematology (Am Soc Hematol Educ Program) 9:207-215, 2004
6. Disis ML, Bernhard H, Shiota FM, et al: Granulocyte-macrophage colony-stimulating factor: an effective adjuvant for protein and peptide-based vaccines. Blood 88:202-210, 1996

**7.** Dranoff G, Jaffee E, Lazenby A, et al: Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proc Natl Acad Sci U S A 90:3539-3543, 1993

**8.** Soiffer R, Hodi FS, Haluska F, et al: Vaccination with irradiated, autologous melanoma cells engineered to secrete granulocyte-macrophage colony-stimulating factor by adenoviral-mediated gene transfer augments antitumor immunity in patients with metastatic melanoma. J Clin Oncol 21:3343-3350, 2003

**9.** Slingluff CL Jr, Petroni GR, Yamshchikov GV, et al: Clinical and immunologic results of a randomized phase II trial of vaccination using four melanoma peptides either administered in granulocyte-macrophage colony-stimulating factor in adjuvant or pulsed on dendritic cells. J Clin Oncol 21:4016-4026, 2003

**10.** Luiten RM, Kueter EWM, Mooi W, et al: Immunogenicity, including vitiligo, and feasibility of vaccination with autologous GM-CSF-transduced tumor cells in metastatic melanoma patients. J Clin Oncol 23:8978-8991, 2005

**11.** Hsueh EC, Essner R, Foshag LJ, et al: Prolonged survival after complete resection of disseminated melanoma and active immunotherapy with a therapeutic cancer vaccine. J Clin Oncol 20:4549-4554, 2002



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