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EDITORIAL

When Will Melanoma Vaccines Be Proven Effective?

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For more than 40 years, investigators have provided countless rationales for the use of cancer vaccines in the prevention and treatment of human cancer [1,2]. No disease has been more targeted for active specific immunotherapy than melanoma. This fascination with melanoma has grown out of a variety of observations, including the rare occurrence of spontaneous clinical remissions, the frequent infiltration of lymphocytes at the primary site of the cancer and its potential prognostic significance, the evidence for objective tumor regressions with cytokines such as interferon alfa and interleukin-2 (presumably through an immune mechanism), and the isolation of functional T cells that recognize melanoma antigens from melanoma patients that can be activated by the same tumor stimuli [3-6]. In addition, there is the virtual absence of any effective nonsurgical treatment for disseminated disease [6a]. During this same time frame, our knowledge of cellular and molecular immunology has progressed from the simple description of different immune cells, such as monocytes and lymphocytes, to the identification of numerous cancer-associated antigens, the T cells that recognize them, and the antigen-presenting cells (APC) that present these cancer antigens to the T lymphocyte [2]. The depth of our understanding and extent of our technical ability now makes it feasible to isolate peptide fragments derived from proteins present within cancer cells that are bound to the major histocompatibity complex (MHC) and presented to the T-cell receptor binding complex on cognate T cells. We now understand the critical role of other cell-surface ("co-stimulatory") molecules on both the APC and the T lymphocyte [7]. Finally, we understand better the most effective APC, the dendritic cell, and the importance of its state of maturation to an effective immune response [8].

The disheartening part of these exciting advances in our understanding of the tumor-specific immune response is the lack of corresponding progress in developing vaccines with proven clinical efficacy against melanoma or any other form of cancer [1]. This should be qualified by acknowledging the advances in vaccine development against two cancer-causing viruses—human papilloma virus (type 16) and hepatitis B. Vaccines against these viruses demonstrate that they may prevent development of cervical cancer and hepatocellular cancer, respectively [9,10].

The underlying reasons for this glaring clinical deficiency is likely not due to the lack of cancer-associated antigens to target or the absence of T lymphocytes that can recognize the cancer antigen–MHC complex through their T-cell receptor. Rather, in patients with advanced cancer, the problem is likely attributable to a dysfunctional host immune response secondary to the tumor's "evade and escape" tactics [11-13]. However, those tactics are not as obvious an explanation for our inability to demonstrate clinically effective vaccines in the adjuvant setting following surgical resection.

At present, there is a large body of literature related to the adjuvant use of several different melanoma vaccines. The publication by Berd et al [14] in this issue of the *Journal of Clinical Oncology* retrospectively summarizes their extensive and unique experience with dinitrophenyl- (DNP-) modified autologous melanoma vaccines after complete surgical resection of bulky regional lymph node disease. Their treatment of 214 melanoma patients throughout 10 years is a very impressive accomplishment. But do these vaccines work?

In this nonrandomized experience, the 214 patients treated with various vaccination schedules had a 5-year relapse-free survival of 33% and an overall survival of 44%. Considering that all patients had to have palpable nodal

disease (N2 or N3 in the current American Joint Committee on Cancer staging system), these results are better than what many might have expected [15]. The results are not broken down according to American Joint Committee on Cancer substage, but even if they were, a historical control group can never take into account all of the subtle selection biases that are associated with recruitment into a clinical trial. Nonetheless, the issue of whether or not the group as a whole fared better than it would have in the absence of vaccination is a critical, but unsolvable question. The importance of this question is magnified by the authors' most interesting and potentially important observation of an association of overall survival, relapse-free survival, and post-relapse survival with the induction of a positive delayed-type hypersensitivity (DTH) skin test to unmodified autologous melanoma. Recall that the patients in this series were vaccinated with modified (haptenated) autologous tumor cells, so any immunologic responses to the vaccine itself could be directed at the hapten and not result in a meaningful antitumor response. So induction of any type of immunity to the unmodified tumor is a very important "intermediate end point" on the path to improving outcomes.

Without knowing for certain that vaccination indeed did improve outcome, the significance of this "intermediate end point" is unknown. One frequently cited explanation for a correlation between immunologic response and improved outcome is that immunologic "responders" are inherently better (usually interpreted as "healthier") than their nonresponder counterparts. In this context, response to vaccination merely acts as a predictor of outcome, perhaps showing us whose antitumor immune response is more intact, and therefore allowing us to guess who will do well before the actual demonstration of clinical disease progression. What makes Dr Berd's experience so interesting in this regard is the evidence against a depressed immune response in his "nonresponder" group. Nearly all patients displayed reactivity to PPD (due to the administration of bacille Calmette-Guerin (BCG) as an adjuvant during the vaccination process) as well as the DNP-modified autologous melanoma.

Could it possibly be that the expression of recognizable antigens on a tumor is inherently associated with a less aggressive malignancy? If so, those tumors recognized by host T lymphocytes simply express antigens that are associated with an improved prognosis. Certainly, in general terms, many known tumor-associated antigens are "differentiation" antigens, more likely to be expressed on well-differentiated (ie, less aggressive) neoplasms. So, this may represent a fundamental principle broadly applicable to many tumor types.

Recent laboratory evidence has shed some light on mechanisms linking antigen expression and outcome in the case of melanoma. Using DNA microarray technology, Bittner et al [16] showed that melanomas expressing high levels of melanoma antigen recognized by T cells (MART-1), a prominent melanocyte lineage antigen, and low levels of expression of Wnt5a, had an inherently less aggressive growth pattern in vitro. A follow-up investigation by Weeraratana et al [17] found that expression of Wnt5a was associated with melanomas of higher histologic grade. Now, preliminary evidence from RNAi experiments shows that Wnt5a may actually directly contribute to the regulation and suppression of MART1 expression. What does all this mean? It may mean that more aggressive melanomas, in this case those that overexpress Wnt5a, downregulate expression of antigens that we know the immune system can respond to and recognize. A measurable but ultimately nontherapeutic immune response, perhaps such as the DTH response to unmodified tumor seen by Berd et al, could indeed be more likely to occur in patients with less aggressive, more differentiated, and more immunogenic tumors. Thus, the immune response would correlate with outcome, without necessarily causing improved outcome.

The reader should recognize that this alternative explanation is just as speculative as the possibility that a subset of vaccinated patients are being helped by the vaccine, because we simply do not know what the untreated outcome for these patients would have been. Also, it should be borne in mind that the two "alternatives" are not mutually exclusive: it may be that we can improve overall outcome by causing a therapeutic immune response that is restricted to a subset of patients (even if it may be an inherently favorable subset), while we need to use more aggressive therapies to impact other subsets. In this regard, the results of a randomized, controlled trial of an allogeneic melanoma cell lysate vaccine (Melacine) are of interest. This vaccine had little overall impact on relapse-free or overall survival [18], but a subset analysis based on patient MHC antigen expression (incontrovertibly a host, rather than a tumor-related, factor) showed that patients who expressed the class I MHC antigens histocompatibility leukocyte antigen–(HLA-)A2 and/or HLA-C3 had improved relapse-free survival [19] and improved overall survival [20] compared to control arm patients bearing the same haplotype, and compared to vaccine arm patients not expressing those HLA antigens. Importantly, HLA status of the control arm patients was not correlated with outcome, so we know in this case that the HLA-A2/C3 haplotype in question was not simply a marker of improved outcome.

So where does this leave us? Berd's provocative data should not be discounted, rather it should serve to remind us how complex the antitumor immune system is, and how little we know about what Berd terms the "immunopharmacology" of tumor vaccines. Optimization of vaccine strategies using definable intermediate end points like DTH response to unmodified tumor, as well as more elegant but less well-understood end points such as Enzyme-Linked

ImmunoSPOT, flow cytometry for cytokine-secreting cells, or tetramer-positive T lymphocytes, can and must take place in phase II trials such as those conducted by Berd [14], Haigh et al [21], Hsueh et al [22], and many others. But, given the unknown variables that could influence these intermediate end points, strong consideration should be given to conducting at least randomized phase II trials of vaccines, so we know with greater certainty that any observed differences between vaccination strategies are likely the result of those strategies and not unanticipated differences among the populations of successive clinical trials. But it remains for large-scale, randomized phase III trials to answer the ultimate question: does any available vaccine "work?"

Unfortunately, there is cause for concern about the feasibility of conducting large-scale phase III trials of melanoma vaccines. A phase III trial comparing vaccination with DNP-modified autologous melanoma to the current standard of high-dose interferon-alfa was halted because of issues relating to the manufacture of the vaccines. Morton's two trials of an allogeneic, whole cell vaccine plus BCG versus BCG alone are ongoing, but accrual of new patients to these trials was suspended for almost a year while manufacturing processes were defined to the satisfaction of the Food and Drug Administration. A confirmatory trial designed to verify the impact of the allogeneic melanoma lysate, Melacine in HLA-A2 and/or HLA-C3 expressing patients with intermediate-risk melanoma has been approved, but has not begun because of a combination of financial and regulatory concerns. The unique issues that confront clinical trials aimed at determining whether melanoma vaccines really can reduce the risk of melanoma recurrence after surgery have not been adequately addressed by researchers, manufacturers, or regulators. This state of affairs can and must change, if we are to build on the efforts of dedicated clinical immunotherapists like David Berd, Don Morton, Malcolm Mitchell, and so many others dating back to William Coley in the late 1800s.

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