Tumor-Specific T-cell Help Is Associated with Improved Survival in Melanoma

Katherine Woods and Jonathan Cebon

Despite success with immune checkpoint inhibitors, clinical benefit from cancer vaccines remains elusive. Combined targeting of melanoma-specific CD4+ and CD8+ T-lymphocyte epitopes was associated with improved survival compared with targeting either alone, or when a nonspecific helper epitope was used. We discuss the potential role of antigen-specific CD4 help. Clin Cancer Res; 19(15); 4021–3. ©2013 AACR.

In this issue of Clinical Cancer Research, Slingluff and colleagues report the results of a phase II clinical trial for melanoma using vaccination with multiple peptide epitopes designed to stimulate both CD8+ cytotoxic T-lymphocyte (CTL) and CD4+ helper T-lymphocyte (HTL) responses (1). The presence of vaccine-induced melanoma-specific HTL responses was associated with increased overall survival. This encouraging result highlights the potential importance of antigen-specific CD4+ T lymphocytes.

Immunotherapy for the treatment of melanoma is advancing in leaps and bounds. Yet despite recent excitement for cancer immunotherapy, particularly with immune checkpoint inhibitors such as anti-CTLA4, anti-PD-1, and PD-L1 (2–5), there has been pessimism about the value of antigen-specific approaches through vaccination (6). Objective responses to vaccination have generally been rare and some reports go so far as to suggest that vaccines may even be harmful (7). In contrast, the present report from Slingluff and colleagues offers encouragement. It shows a clear signal of clinical benefit using a strategy that aims at stimulating antigen-specific CD4+ T lymphocytes (1).

In the study, multipeptide melanoma vaccines that stimulate CD4+ HTL were evaluated for augmentation of CD8+ CTL responses. Cohorts received peptide mixtures that were the targets for CD8+ T-cell responses, CD4+ responses, or both. The CD4 epitopes were either melanoma-specific or against a CD4 epitope derived from tetanus toxoid and therefore not expressed on tumor. Although the vaccine regimens were all immunogenic, the helper peptides did not augment detectable CTL responses. Nonetheless, there was a strong and significant association between survival and responses to a panel of 6 melanoma helper peptides. This effect was not seen following injection of the tetanus helper peptide.

As randomization in this study had no effect on outcome, it cannot be assumed that the intervention and subsequent HTL responses mediated this improved survival. For instance, the HTL responses might reflect greater immune “fitness” and identify patients with more responsive immune systems due to unrelated mechanisms (such as fewer regulatory cells, myeloid-derived suppressor cells, or a less immunosuppressive tumor microenvironment). Such patients might have had a better chance of survival independently of any treatment. Nonetheless, no such benefit occurred in association with tetanus peptide responsiveness, so it seems likely that the specificity of the peptides was important.

The explanation for clinical impact here is a matter for speculation and this study raises more questions than it answers. Nonetheless, it points to a potentially important difference between melanoma-specific and nonspecific T-cell help. Figure 1 outlines an overview of the potential mechanisms by which induction of HTL specific for melanoma epitopes may confer the observed survival advantage, and compares this with induction of HTL specific for a nontumor epitope. These are further discussed below.

Helper responses can be expected to enhance CTL proliferation following vaccination by activating local antigen-presenting cells (APC). This can be mediated by cytokine production or direct interaction between CD40L and CD40 on APC (8). Thus, activated APC will be better equipped to stimulate CD8+ T-cell responses. Locoregional effects at the vaccine site cannot, however, explain why melanoma-specific class II-restricted peptides should work more effectively than a tetanus-derived antigen, especially as most, if not all, recipients will have had prior exposure to tetanus. As a result, they could be expected to mount a brisk response from preexisting memory CD4+ T-cells, which could populate vaccine sites and help drive CTL proliferation.

Although dogma states that helper cells work by enhancing CTL responses, no increase in circulating CTL was seen. Indeed, if anything, there was a drop in CTL responders following vaccination.
when HTL peptides were added (28% vs. 43% of patients when CTL peptides were used alone). This effect was not observed with the tetanus helper peptide. Admittedly, this may not necessarily reflect the total CTL pool and could reflect a shift in cell localization, that is, helper responses facilitated egress of CD8\(^+\) effectors, say to antigen sites, thereby reducing circulating numbers. This was not established in the current study and would require biopsies or tracking studies to evaluate further.

An alternative explanation is that HTL localized into the tumor and induced microenvironmental changes that led to enhanced patient survival, presumably as a consequence

### Figure 1.

Tumor-specific versus nonspecific help. Following vaccination peptides are delivered to APC in regional lymphoid tissue. These are taken up and presented: short class I-binding peptides in association with HLA class I, longer class II-binding epitopes in association with HLA class II. Priming phase: when circulating antigen (Ag)-specific CD4\(^+\) or CD8\(^+\) lymphocytes encounter melanoma-specific Ag in association with HLA, binding occurs. In the presence of appropriate costimulation clonal expansion occurs. CD4\(^+\) cells also provide help by stimulating DC to upregulate CD40L and by secreting cytokines such as interleukin 12 (IL-12) and IFN\(\gamma\). Immune activation is tempered by counter-regulatory mechanisms such as upregulation of CTLA4, a process that can be targeted using immunotherapeutic antibodies. Help for B cells may also facilitate antibody production. Circulation and localization: antigen-specific lymphocytes generated following vaccination enter the circulation and localize to wherever antigen is presented. T cells that recognize targets that are presented in the tumor may localize, those recognizing irrelevant targets such as tetanus peptide will not. Within tumor: CD8\(^+\) and CD4\(^+\) T lymphocytes recognizing tumor Ag may localize into the tumor where their fate will depend on whether or not the environment is receptive. The tumor may evade or subvert immunity by downregulating HLA class I, through the secretion of immunosuppressive cytokines such as IL-10 or TGF\(\beta\) and by engaging regulatory immune checkpoints; programmed cell death ligand 1 (PD-L1) by tumor cells or programmed cell death ligand 2 (PD-L2) by tumor-associated APC. CD4\(^+\) cells may bind to antigens presented within the tumor: melanoma can express HLA class II, so direct binding may occur to tumor cells and/or intratumoral stromal APC. This can result in cytokine secretion, antagonism of regulatory mechanisms, and as CD4\(^+\) cells may also act as effectors, direct cytotoxicity. Modification of the microenvironment may facilitate CTL access and killing. Nontumor-specific help tetanus-specific HTL will not encounter ligand within the tumor as ligand (HLA-peptide complex) is absent.
of immune effects. Here, they could bind to melanoma-specific peptides presented on intratumoral APC in association with HLA class II complex. In addition, melanoma cells frequently express the MHC class II complex (9), so there is also the potential for direct presentation of these peptide antigens by tumor. In either case, the CD4+ lymphocytes might be expected to respond by producing cytokines, by proliferating, or even by directly killing, tumor cells. Previous studies have shown that the HTL peptides used by the authors induced Th1 dominant responses (1, 10). Infiltrating CD4+ cells could therefore also increase the CD4+ effector:Treg ratio, reduce intratumoral immunosuppression, and facilitate effector CTL responses (11).

As no enhancement of vaccine-induced CTL responses was seen, another possibility worth considering is that intratumoral inflammation stimulated endogenous immunity. This may have induced CTL responses against a variety of epitopes that were not included in the vaccine pool and therefore not subject to analysis by the immune monitoring strategy described herein. Similarly, CD4+ T lymphocytes play a role in the stimulation of B lymphocytes and antibody production (8), which might have also contributed to survival. No antibody responses were sought, so any potential role remains unknown.

It is an exciting time in the field of melanoma immunotherapy and great progress is being reported. Nonetheless, approaches remain largely empirical despite a strong desire for more detailed understanding of mechanisms. These are often complex and explanations for improved clinical outcomes are based on speculation that cannot be defined precisely despite immune monitoring. Biopsies and cytotoxicity assays will help understand the possibilities in greater detail but will still be imperfect. Nonetheless, combination treatment approaches are in the pipeline and the most effective combinations will be those that enable complementary mechanisms to be added or to synergize. Vaccines and immune checkpoint inhibitors need to be combined, and when designing such trials further investigation of antigen-specific help is certainly warranted.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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