The prognostic impact of specific CD4 T-cell responses is critically dependent on the target antigen in melanoma

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Many clinical trials now demonstrate that cancer immunotherapy can treat melanoma and other cancers previously considered refractory to therapies other than surgery. Enhancing tumor antigen-reactive autologous T-cell responses by adoptive transfer of tumor-specific T-cells, or administering immune checkpoint inhibitory antibodies and/or use of cancer vaccines can result in impressive clinical responses.1,2 However, clinical benefits are commonly limited to a subset of patients and biomarkers predicting responsiveness are lacking. The pattern of T-cells infiltrating resected primary colon cancer has been shown to be informative for patient survival thereafter,3 suggesting that the continued presence of these cells in the periphery is of crucial importance for long-term outcome. Biomarkers predicting patient survival that were based on peripheral blood analyses would not require biopsies of resected tissue, would be easier to offer as routine and would allow serial studies more conveniently. Initially, we reported that the presence of peripheral T-cells reacting to tumor-associated antigens in unresectable stage IV melanoma patients, had a strong positive impact on survival. Patients possessing T-cells targeting the two common melanoma-associated antigens NY-ESO-1 and Melan-A, but not survivin or MAGE-A3, had a longer median survival and a higher 1-year survival rate than those without such T-cells.4 In this first analysis, we stratified patients simply on the basis of whether they possessed either or both CD4+ or CD8+ T-cells responding to tumor antigens by producing any one of the six cytokines we measured (IFN-γ, IL-2, IL-4, IL-10, IL-17, TNF). In a more recent analysis, we determined in detail whether survival was different in patients depending on the phenotype (CD4+ vs. CD8+) and the nature of the cytokine-response (pro- vs. anti-inflammatory cytokines).5

Our investigations revealed that the distribution of T-cell responses to NY-ESO-1 vs-Melan-A was markedly different, and this influenced survival. Concerning NY-ESO-1, we observed that a majority of patients (67%) possessed CD4+ T-cells targeting this particular antigen. A solely CD8+ T-cell respond was seen only in 9%, and both CD4+ and CD8+ T-cells in 24%. Reactivity against NY-ESO-1 was always associated with a survival benefit, whether it was mediated by CD4+ or CD8+ T-cells alone or both together. In contrast, for reactivity against Melan-A, 34% of patients showed a purely CD4+ response and 40% a CD8+ response (with 26% having both).

The presence of CD8+ Melan-A-reactive T-cells was also associated with longer survival but unlike NY-ESO-1 reactivity, the presence of CD4+ T-cell responses to Melan-A were not associated with a clinical benefit. These patients’ median survival time was similar to patients without Melan-A-specific T-cells, even when they also possessed CD8+ T-cells reacting to this antigen. Thus, it appears that the presence CD4+ T-cells targeting Melan-A had a dominant negative effect, suggesting that they were suppressive. Interestingly, analysis of cytokine production by Melan-A-reactive CD4+ T-cells revealed that only those positive for IL-4 and IL-17 were negatively associated with survival, and this were not likely to be “classical” Tregs.

Although we had previously shown that the frequency of circulating CD4+CD25+ FoxP3+ Tregs did not correlate with survival in late stage melanoma patients,6 accumulating data show that Tregs can act in an antigen-specific manner,7 suggesting that an association between overall frequency and survival would not necessarily be observed. Antigen-specific Tregs are more likely to mediate their suppressive activity by cell-cell contact rather than by secretion of soluble factors.8 Hence we would not have detected these in our assays. Unfortunately, data on Foxp3 expression is not available for this study, making it difficult to estimate the...
proportion of classical Tregs among the Melan-A-responsive CD4\(^+\) T-cells.

In contrast to Melan-A reactivity, the presence of CD4\(^+\) Melan-A-specific T-cells is not associated with prolonged survival. Possible explanation: double negative (DN) thymocytes migrate from the bone marrow to the thymus. Thymus epithelial cells (TECs) present autologous peptides to thymocytes in order to eliminate self-reactive T-cells during negative selection. Self-tolerant, high avidity naïve T-cells leave the thymus and migrate to secondary lymphoid organs. If self-reactive T-cells are not completely eliminated, peripheral tolerance mechanisms (e.g., Tregs) prevent autoimmunity.

![Figure 1](image.png)

**Figure 1.** The distribution of T-cell responses to NY-ESO-1 and Melan-A is different, and presence of CD4\(^+\) Melan-A-specific T-cells is not associated with prolonged survival. Possible explanation: double negative (DN) thymocytes migrate from the bone marrow to the thymus. Thymus epithelial cells (TECs) present autologous peptides to thymocytes in order to eliminate self-reactive T-cells during negative selection. Self-tolerant, high avidity naïve T-cells leave the thymus and migrate to secondary lymphoid organs. If self-reactive T-cells are not completely eliminated, peripheral tolerance mechanisms (e.g., Tregs) prevent autoimmunity.

In order to prevent autoimmunity is not required. In contrast, the differentiation antigen Melan-A is constitutively expressed by normal melanocytes as well as melanoma cells, so T-cells directed against it should be deleted during negative selection. However, there is broad evidence that this process is incomplete and Melan-A-specific T-cell responses can be observed also in healthy subjects.\(^9\) Thus, peripheral tolerance mechanisms are assumed to be of critical importance to prevent autoimmunity (Fig. 1).

Taken together, our results confirm the significance of NY-ESO-1 in melanoma immunity, with responses by both CD4\(^+\) and CD8\(^+\) T-cells beneficial for patient survival. The fact that more patients showed CD4\(^+\) responses against NY-ESO-1 provides another rationale to activate NY-ESO-1-specific Th1-responses in the therapeutic setting, as successfully demonstrated before.\(^1\)

Nevertheless, the activation of antigen-specific CD4\(^+\) T-cells by immunotherapy might also harbor some risks. Th1 responses are assumed to be beneficial while the activation of Tregs might be disadvantageous especially if constitutively expressed self-antigens are targeted. Furthermore, clinical approaches that induce both CD4\(^+\) and CD8\(^+\) responses, such as whole protein vaccination, may be counterproductive depending on the nature of the T-cells induced. Hence, vaccination with MHC class I epitopes or adoptive T-cell transfer of previously-generated, antigen-specific CD8\(^+\) T-cells might be the most promising approach for inducing favorable immune responses against Melan-A.

The present work compared only Melan-A and NY-ESO-1 as representatives of these two antigen types. Clearly there are many more antigens targeted in immunotherapy and immunosurveillance, and dissection of their ability to induce different T-cell responses would be valuable in future studies.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
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