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Title: Analysis of the neo-antigen specific T cell response: and consequences for personalized immunotherapy
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Biomarkers in Cancer Immunotherapy

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Antibodies against T cell checkpoint molecules have started to revolutionize cancer treatment. Nevertheless, less than half of all patients respond to these immunotherapies. Recent work supports the potential value of biomarkers that predict therapy outcome and inspires the development of assay systems that interrogate other aspects of the cancer-immunity cycle.

Developments in T cell checkpoint-based cancer immunotherapies are occurring at a mind blowing pace. Following the demonstration of improved survival in melanoma patients treated with anti-CTLA4, the clinical value of targeting the PD-1 axis has been demonstrated in tumor types as diverse as melanoma, non-small cell lung cancer, bladder cancer, and Hodgkin’s lymphoma. Although these clinical data are extraordinary, the dearth of biomarkers that can identify those patients who are most likely to benefit from these therapies forms a limitation. Identification of such biomarkers would avoid treatment-related toxicity and cost in patients who are unlikely to benefit, and should also increase our understanding of modes of action of immunotherapy and thereby identify potential combination therapies.

A recent publication describes a genetic basis for clinical response to CTLA-4 blockade in melanoma patients, allowing the discrimination of responding from non-responding patients prior to therapy (Snyder et al., 2014). Furthermore, work by a number of groups describes aspects of the tumor micro-environment that may be of value to predict response to blockade of the PD-1 – PD-L1 axis (Herbst et al., 2014; Powles et al., 2014; Tumeh et al., 2014).

Mutated peptides resulting from DNA mutations are recognized by CD8+ (Robbins et al., 2013; van Rooij et al., 2013) and CD4+ T cells (Linnemann, 2014) in a large fraction of melanoma patients, and these neo-antigen specific T cell responses are likely to contribute to the clinical effects of cancer immunotherapy (Heemskerk et al., 2013). To analyze potential effects of cancer genomes on response to immunotherapy, Snyder and colleagues characterized the tumor mutational landscape from melanoma patients treated with the anti-CTLA-4 antibody ipilimumab. Within both a discovery and validation cohort, a higher mutational load was associated with long-term benefit. Overall survival was significantly higher in patients with a high mutational load in the discovery cohort, and a non-significant trend towards improved survival was seen in the validation cohort. These data provide some support for the hypothesis that immunotherapy is particularly effective in highly mutagenized tumors, but analysis of larger cohorts will be required to firm up this conclusion.
Next, the authors developed a bioinformatics pipeline to predict the neo-epitopes that may be recognized by CD8+ T cells, as based on predicted binding to patient-specific HLA class I alleles, predicted immunogenicity, and similarity to known T cell epitopes. Strikingly, the authors subsequently identified 101 four amino acid (tetrapeptide) motifs within the predicted nonameric neo-epitopes that were shared between patients with a long-term clinical benefit but absent in tumors of patients with no or minimal clinical benefit. Furthermore, simulations using five models showed that the observed correlation between the identified signature and clinical course was very unlikely to have arisen by chance. Perhaps most strikingly, the predictive value of this signature was confirmed in an independent validation cohort.

How may the predictive value of the identified tetrapeptide motifs be explained? On the basis of their data, Snyder and colleagues propose that mutated peptides that contain one of these tetrapeptide motifs are targets for autologous T cells. In support of this, the authors document T cell reactivity against two such peptides following in vitro restimulation of autologous T cells. Nevertheless, a number of aspects of the proposed role of tetrapeptide containing epitopes as cancer rejection antigens remain to be elucidated. First, while different HLA alleles can bind peptides with similar sequences, the observation that the tetrapeptide motifs do not show an HLA restriction is unexpected. Second, a given tetrapeptide sequence is observed to occur in different positions in different mutated peptides, arguing against a fixed role of the tetrapeptide motif in TCR recognition. It may be reasoned that the human TCR repertoire is biased towards recognition of certain motifs that are ‘foreign’, and some of the motifs identified by Snyder and colleagues are enriched in known T cell epitopes derived from human pathogens. However, as similarity to known T cell epitopes is part of the bioinformatics pipeline used to identify candidate neo-epitopes, this is perhaps not entirely unexpected. As a side note, to determine whether the identified motifs may be characteristic of non-human genomes, we calculated the occurrence of the tetrapeptide motifs in the human genome versus a series of bacterial genomes (n = 14). This analysis provides little evidence for substantial overrepresentation of the identified tetrapeptide motifs in non-human genomes (unpublished results, C. Kesmir).

If the human neo-antigen specific T-cell response would be focused on mutated peptides that contain one of the tetrapeptide motifs, one would expect such motifs to be frequently observed in validated neo-antigens identified in other studies. Analysis of the occurrence of the tetrapeptide motifs in a set of nonameric neo-antigens in human melanoma (Robbins et al., 2013; van Buuren et al., 2014; van Rooij et al., 2013) shows that 1 out of 13 neo-antigens contained one of the 101 tetrapeptide motifs (Figure 1A, left). As a
comparison, the frequency of these motifs within nonameric peptides that are predicted to bind to HLA class I in the entire human genome is approximately 2% (Figure 1A, right). Obviously, the list of documented neo-epitopes is presently too small to tell whether tetrapeptide motifs occur more often in melanoma neo-antigens than would be expected by chance. However, this analysis does suggest that T cell recognition of mutated epitopes that contain one of the identified tetrapeptide motifs is not a dominant component of the neo-antigen specific T cell response in human melanoma. It will be exciting to further dissect by which mechanism the occurrence of the tetrapeptide signature predicts the likelihood of response to CTLA-4 targeting in future studies.

The data from Snyder and colleagues provide strong evidence that genomic characteristics of human tumors can form potential biomarkers in cancer immunotherapy. At the same time it is apparent that information on other aspects of the interaction between the T cell based immune system and tumor cells (‘the cancer-immunity cycle’ (Chen and Mellman, 2013)) will provide relevant information. Using cancer cells as a starting point (Figure 1B), a first set of biomarkers can be envisioned that describe whether the tumor is likely to contain antigens that may be recognized by T cells, be it reflected by either the size of the foreign antigen space generated by mutations and viral antigens, or the type of signature described by Chan and colleagues. Second, it may be useful to consider biomarkers that provide a simple measure of general immune status, thereby indicating whether a patient has the capacity to mount a relevant T cell response. Third, the accumulation of a CD8+ T cell pool within the tumor and a bias of this T cell pool towards a restricted number of TCR clonotypes is likely to provide substantial predictive value, as demonstrated by Ribas and colleagues in melanoma patients treated with anti-PD1 (Tumeh et al., 2014). Finally, expression of inhibitory molecules such as PD-L1 within the tumor micro-environment can both provide information on an ongoing immune dialogue and on potential targets for therapeutic intervention. Indeed, expression of PD-L1 on tumor infiltrating immune cells has recently been put forward as a possible biomarker in anti-PD-L1 treatment (Herbst et al., 2014; Powles et al., 2014).

Looking forward, a coordinated effort to assess the value of both genetic and non-genetic biomarkers that address different aspects of the cancer-immunity cycle in T cell checkpoint blockade will allow the field to integrate information on individual aspects of tumor-immune interaction. In addition, such analyses should yield biomarkers that - either alone or in combination - will allow the development of rationally designed combination therapies for individual patients.
Figure 1. Neo-antigen signatures and other biomarkers within the cancer-immunity cycle.

(A) The percentage of nonameric peptides containing one of the tetrapeptide motifs within either documented neo-epitopes (left, n = 13), or nonameric peptides derived from the human proteome predicted to bind with an affinity of ≤ 500nM to HLA-A*01:01, A*02:01, A*03:01, B*07:02 or B*08:01 (right, n = 2,327,696). Blue chart piece represents the percentage of epitopes containing one of the motifs.

(B) Opportunities for biomarker discovery (blue) in the cancer-immunity cycle.


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References


