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Immune signatures predicting responses to immunomodulatory antibody therapy

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Since the first immunomodulatory antibody was licensed by the FDA in 2011 for treating melanoma it has remained the case that only a certain proportion of cancer patients respond favourably to a particular therapy. Recent results from combining two or more different antibodies each targeting a different immune checkpoint indicate that the proportion of responding patients can be increased, but thus far there are no such therapies routinely yielding clinical benefit in 100% of patients in any cancer type. Therefore, predicting which patients will respond to a particular therapy remains of the utmost importance in order to maximise treatment efficacy and minimise side-effects and costs. Moreover, determining biomarkers predicting responses may provide insight into the mechanisms responsible for success or failure of that therapy. This article reviews seminal papers mostly from the past two years of progress in this area of intense investigation, and mostly in melanoma, the tumour type for which the largest body of data exists thus far.

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Introduction

Many early immunotherapy trials predate the advent of 'checkpoint blockade' (i.e. the use of antibodies to disrupt interactions between immune cell receptors which deliver down-modulatory signals and their ligands on cancer cells or other cells in the tumor). For several reasons, these early attempts to harness immunological anti-cancer activities commonly targeted metastatic melanoma not only because of the lack of effective conventional treatments but mostly because of the belief that melanoma was more immunogenic than other tumours. The rare occurrence of spontaneous regressions, accompanied by vitiligo, was thought perhaps to represent a special case of the anti-cancer effects of autoimmunity, which would therefore not be relevant for most other tumours [1]. Thus, because many early immunotherapy trials focussed on melanoma, this may explain why the first and still most extensive clinical experience with checkpoint blockade was also gained in this tumor type. For historical reasons, many of the first data on biomarkers predicting responses to immunomodulatory antibody therapy were from trials of the anti-CTLA-4 (CD152) monoclonal antibodies (mAbs) ipilimumab or tremelimumab in metastatic melanoma. Although this target remains of interest, in the meantime a much larger body of data is accumulating on the use of anti-PD-1 (CD279) and PD-L1 (CD274) antibodies not only in melanoma but in many other solid cancers as well [2]; indeed there are already strong arguments for using anti-PD-1 mAb as first-line therapy for non-small cell lung cancer, with results similar to chemotherapy but with fewer serious side effects [3,4]. Informative biomarkers for response to PD-1/PD-L1 blockade, which may be different in different tumour types, are now beginning to emerge, as are predictive biomarkers for responses to combination therapy targeting both the CTLA-4 and PD-1 pathways. Finally, trials of immunomodulatory antibodies targeting one or other of the many additional immune checkpoints (for recent review, see [5]) are also underway. Additionally, agonist immunomodulatory antibodies are also becoming a focus of interest in the cancer context [6,7]. Here, for reasons of space constraints, recent publications on biomarkers relevant to anti-CTLA-4, anti-PD-1 and anti-PD-L1 immunotherapies will be reviewed and future prospects considered.

Immune signatures predicting responses to anti-CTLA-4 antibody therapy

Two antibodies targeting CTLA-4 were extensively tested in the earliest clinical trials of checkpoint blockade in metastatic melanoma, following the demonstration of activity of CTLA-4 blockade in a small number of patients with melanoma [8] and ovarian cancer [9]. After the long interregnum required to measure efficacy in terms of overall survival (OS) rather than the less-reliable measure of progression-free survival (PFS) as pointed out by Tan *et al.* [10], one CTLA-4 mAb (Tremelimumab) failed to

improve OS relative to standard chemotherapy in the crucial phase III trial [11]. Fortunately, the other mAb (Ipilimumab) had already been shown to extend OS in two phase III trials [12,13], leading to its licensing by the FDA in 2011. These findings illustrate an important point, namely that properties and nature of the mAb used most likely critically influence clinical outcome even when the target molecule is identical. Thus, biomarkers predicting response to therapy may be specific not only for the target antigen and tumor type but for the antibody itself. For Ipilimumab treatment of stage III or IV melanoma, a survival analysis of pooled data from 12 trials yielded a median OS of 11.4 months [14]. This is in fact not much better than the 8–10 months achieved with other, conventional, therapies. However, the survival curve plateaued at around 20% after 3 years, twice the 10% achieved with conventional therapies [15]. Moreover, some patients had survived an unprecedented 10 years or more [14].

Clearly these striking results immediately raised the question of predictive biomarkers and mechanisms explaining differential survivals. Two main approaches can be taken to this answering this question: what can be construed from any data acquired using the resected tumor itself and what can be concluded from analyses of peripheral blood. From the practical point of view, resected specimens are not always available and the ability to monitor patients using serial biopsies is challenging and potentially hazardous to the patient. Assays performed on blood are essentially non-invasive, convenient for routine testing and can be repeated almost at will. Unsurprisingly, the majority of data on predictive immune signatures for responses to Ipilimumab originate from blood tests. These latter tests can be divided into those performed at baseline, before beginning treatment with Ipilimumab (truly predictive) and those performed during or after treatment (informative for correlates of response or non-response). Such assays are almost impossible using serial biopsies although some efforts describing monitoring changing immune signatures in serially resected deposits after failing treatment with anti-CTLA-4 and during treatment with anti-PD-1 mAbs have been made [16,17]. These are of course not strictly predictive biomarkers in the sense discussed here.

Immune signatures predicting responses to anti-CTLA-4 antibody therapy: intratumoral

The earliest studies on ipilimumab-treated melanoma already provided some striking results, including the counterintuitive finding that a higher baseline presence of FoxP3+ T cells and indoleamine 2,3-dioxygenase, both thought to suppress anti-tumor T cell responses, were associated with clinical benefit [18] but also confirming that high baseline expression of genes implicated in anti-cancer responses were positive predictors of response [18,19]. Melanoma is a tumour with a high degree of somatic mutations, presumably due to UV-irradiation; many other tumours have fewer mutations but the principle of generating neoantigens recognizable by the immune system pertains to all and may be essential for the success of checkpoint blockade. Thus, the earliest studies on this, again in melanoma, showed correlations between baseline actual neoantigen load, but not overall mutational load, with clinical benefit of anti-CTLA-4 treatment [20], which also correlated with the presence of mRNA for the mediators of cellular cytotoxicity granzyme A and perforin [21].

Immune signatures predicting responses to anti-CTLA-4 antibody therapy: extratumoral

Some recent studies have focused on tumor-draining lymph nodes [22] but the majority has been limited to blood tests, which are the most useful for patient monitoring. Most investigations have included baseline samples for prediction of response, and follow-up sampling to assess correlations of ongoing responses. Here we will limit the discussion to baseline predictors of response. Again, most of the data pertain to melanoma patients treated with ipilimumab, predominantly looking at cellular components, but also encompassing soluble factors such as VEGF [23]. It might be considered *a priori* unlikely that a single biomarker with robust predictive attributes could be identified, but a good candidate for such a marker in many studies is the level of so-called myeloid-derived suppressor cells (MDSCs). Baseline values for monocytic MDSCs were reported to be informative for clinical response to ipilimumab in a small study of 49 patients [24], and this was confirmed in a later multi-center study of over 200 patients with validation of the biomarkers [25•]. Moreover, of a total of 28 variables examined in the latter study, a combination of 6 (lactate dehydrogenase, relative lymphocyte count, absolute monocyte count, absolute eosinophil count, frequency of monocytic MDSC and frequency of CD4+ FoxP3+ regulatory T cells) formed a risk score on the basis of which clinical responses to ipilimumab treatment could be predicted, with 40% of patients in the non-risk group surviving >4 years as opposed to no 4-year survivors in the risk groups [25•]. A little-suspected prominent role for eosinophils may relate to findings in mice that eosinophils enhance infiltration of CD8+ T cells into the tumor, possibly associated with their effect on normalizing tumor blood vessels [26]. The inclusion of additional less-well investigated parameters, such as frequencies of T cells bearing γδ-T cell receptors, may further improve the predictive strength of these immune signatures [27] as might more detailed assessment of cell populations such as MDSCs using more sophisticated analytical technologies [28].

Immune signatures predicting responses to anti-PD-1 or PD-L1 antibody therapy

Because anti-CTLA-4 mAb are seldom used as monotherapies in current clinical practice, an important question is whether the predictive biomarkers established for