Commentary

Predicting the response to anti-PD1 therapy in metastatic melanoma

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Checkpoint inhibitor therapy has proven effective in metastatic melanoma and a range of other tumor entities including non-small cell lung carcinoma, Hodgkin lymphoma and renal cell carcinoma. Despite the impressive and often durable induction of tumor regression, 60–70% of patients with metastatic melanoma do not respond to single agent therapy with the anti-programmed death (PD)-1 antibodies pembrolizumab (1) or nivolumab (2). Combination treatment with ipilimumab, an anti-cytotoxic T-lymphocyte-associated protein (CTLA)-4 antibody, has resulted in higher response rates of 58%; however, this benefit comes with around 55% grade 3/4 side effects (2).

Since anti-PD1 treatment induces toxicity and is enormously expensive, and since treatment alternatives exist (at least for a subset of patients), the scientific community is eager to find predictive markers for response. For the prediction of response to a specific therapy, either pretreatment tumor biopsies or pretreatment liquid biopsies should indicate whether a patient is more likely to respond to a specific therapy as compared to an alternative therapy. It is especially important to distinguish positive prognostic factors from positive predictive factors. Patients with a normal lactate dehydrogenase (LDH), for example, show better response rates and longer survival in comparison to patients with elevated LDH for different therapy modalities such as immune checkpoint inhibitors, BRAF inhibitors, or even chemotherapy.

For melanoma, the promises that PD-L1 expression in tumor samples could serve as a predictive marker have been disappointing. The use of PD-L1—although a robust marker in some tumor entities—has been hampered by a number of challenges. First, PD-L1 assessment by immunohistochemistry is not standardized (3,4). Second, PD-L1 expression in melanoma is low, difficult to evaluate and heterogeneous within the tumor (5), across tumor sites as well as over time (6). Last but not least, there are indications that PD-L1 expression in pretreatment biopsies is rather prognostic than predictive, with better survival of PD-L1-positive melanoma patients in patients treated with dacarbazine chemotherapy as well as under anti-PD1 treatment as shown in a randomized study (1,2,7). In advanced melanoma, objective response rates (ORR) for nivolumab were 57.5% and 41.3% for PD-L1-positive and PD-L1-negative tumors, respectively (2). However, even though patients with PD-L1-positive melanoma exhibit higher response rates to anti-PD1 therapy, the majority of responders do not express PD-L1. Therefore, excluding patients with PD-L1-negative melanoma would deprive a substantial number of patients from an effective therapy.

The next potential candidate for a predictive marker of anti-PD1 therapy response was mutational load. A landmark paper showed the different levels of somatic mutations in various cancer entities (8). Subsequently, the mutational load was linked to response to anti-PD1 such that high mutational load correlated with higher response rates (9-11). This fit well for melanoma, which had the highest mutational load and a high response rate to anti-PD1 treatment, as well as for colorectal cancer (CRC), which had very low mutational load and showed low to no response to anti-PD1 antibody therapy (9). Interestingly, in CRC the subgroup of patients with DNA microsatellite instability leading to mismatch-repair deficiency is responsive to anti-PD1, and in lung cancer smoking that induces mutations is a predictor of response (10). However, in Merkel cell

carcinoma, which responds well to anti-PD1 treatment mutational load was not predictive for response. Here, patients with high mutational load showed lower response rates than those with low mutational load (12). The difference may be due to oncoantigen expression in virus-positive and virus-negative tumors, as pre-existing immune responses are known to be a precondition for response (13).

The aim of finding a single factor predictive of response has been replaced by more comprehensive approaches. Hugo *et al*. very broadly analyzed mutanomes and transcriptomes in pretreatment biopsies to address this enormously important question. Even though the number of patients is quite small (with 34 pretreatment biopsies and 4 early on-treatment biopsies of melanoma patients), they composed the groups with 1:1 responders and non-responders, and then investigated the samples including mutational load with whole exome sequencing. RNA sequencing was performed in a subset of 28 pre-treatment biopsies. When analyzing immune-related markers a separate analysis of metastases from different organs – especially when including lymph node metastases- is advisable to check for potential bias as we did in a previous analysis of 178 pretreatment biopsies that found that PD-L1 was not predictive for response to ipilimumab (14). However, Hugo *et al.* do not provide this information on the origin of metastases and consequently disbalance of tumor samples with respect to organ location cannot be excluded.

Interestingly, Hugo *et al.* found that high mutational load was not associated with response but with survival. The patients with a mutational load in the top third survived significantly longer than those with a mutational load in the bottom third. Hugo *et al.* also analyzed markers that had been previously identified. However, as in most biomarker studies, markers that have been identified in one population (discovery set) often cannot be validated in another (validation set). For example, a tetrapeptide signature found by Snyder *et al.* (15) that was associated with response to anti-CTLA4 treatment did not predict response to anti-CTLA4 treatment in another study (16) or to anti-PD1 in the present study.

Concerning non-synonymous single nucleotide variants (nsSNV) and copy number variations, no statistically significant difference was found between responders and non-responders. Only BRCA2 nsSNV were enriched in patients who responded to anti-PD1 treatment, with 6 out of 7 (86%) patients with BRCA2 nsSNV tumor showing a response. However, since the response rate in non-BRCA2 nsSNV tumors was also 15/31 (48%) use of BRCA2 nsSNV as a marker in predicting the response of a specific patient before treatment with anti-PD1 antibody is limited. On the other hand, Hugo *et al.* determined that a specific transcriptional signature referred to as IPRES (innate anti-PD1 resistance) was associated with a poor response to anti-PD1 treatment. This signature mainly includes genes of mesenchymal transition, extracellular matrix remodeling, angiogenesis and wound healing. Most of these genes, including IL-10, VEGFA, VEGFC, CCL2, CCL7, CCL8 and CCL13, have previously been reported as biomarkers in cancer. In mouse models VEGFA and CCL2 were also shown to induce resistance to anti-PD1 (17). Furthermore, acquired resistance to PD1 blockade in patients with melanoma was associated with defects in pathways involved in antigen presentation and interferon receptor signaling (18). The differential expression of mesenchymal genes that was found might really hint at the upcoming knowledge on the importance of the stroma in eliminating tumors. WNT5A that is also part of the IPRES has been known to promote melanoma growth, tumorigenesis, activation of AKT signaling and can enhance resistance of melanoma cells to BRAF inhibitors (19).

Importantly, the Hugo *et al.* study provides further evidence that predictive markers differ between anti-CTLA4 treatment and anti-PD1 treatment. This is in line with the clinical observation that patients can respond to both therapies differentially. Whereas granzyme A and perforin 1, molecules indicative of CD8+ T cell cytolytic activity, have been described with higher expression in patients who respond to CTLA4, these were not found to be elevated in anti-PD1 responders, and the same holds true for the IPRES signature which was not found to be associated with anti-CTLA4 resistance (16). Taken together, these results remind us that cancer, the immune system and potential therapeutic drugs interact in a complex network of biological pathways and it is unlikely there being an easy single marker for response to any one immune therapy. Furthermore, optimal sequencing of therapies could depend on specific signatures. Indeed, mitogen activated protein kinase (MAPK) inhibitor therapy induces similar signatures, suggesting that MAPK-inhibitor resistance mediates cross-resistance to anti-PD1 therapy. In accordance with that, there have been hints that immunotherapy with checkpoint inhibitors might be less effective when used sequentially after progress on targeted therapy in melanoma (20).

Importantly, the authors also validated their IPRES

signature across other tumor entities, a critical point since biomarkers like PD-L1 are predictive for response in non-small cell lung cancer but not in melanoma (21). Since approval for anti-PD1 therapy has been granted for non-small cell lung cancer and kidney cancer and is on the way for other tumor entities, these findings pointing to a universal expression signature are especially important. However, the key in small studies like this one is not to find a marker for individual treatment decisions in single patients but to further characterize mechanisms of action of and resistance to tumor immunotherapy in order to better understand the tumor microenvironment and subsequently derive hypotheses for synergistic treatment combinations.

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Footnote

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