Molecular features of response to anti-PD-1 therapy

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For decades, researchers and clinicians have provided evidence that human malignancies are recognized and controlled by the autologous immune system. By the end of the last century, numerous tumor antigens had been found and T cells capable of lysing tumors in an antigen-specific manner had been isolated (1). However, vaccinations and other types of immunotherapy showed only very limited clinical efficacy.

By the beginning of new millennium, a new class of anti-cancer drugs has constantly worked its way to the center stage of modern oncology: immune-checkpoint blocking agents. Based on the pioneering preclinical work of Jim Allison (2) and Lieping Chen (3), CTLA-4 and PD-1 blocking antibodies have shown their clinical activity in a variety of solid and non-solid tumor. The CTLA-4 blocking antibody ipilimumab was the first immune-checkpoint blocker proving its efficacy in a prospective randomized trial (4). In a pooled analysis of all patients from clinical trials employing ipilimumab as a monotherapy, a 5-year survival rate of approximately 20% was observed with a plateau being reached (5). This indicates that cancer immunotherapy can cause durable clinical benefit in advanced melanoma. For PD-1 blockade, long-term data is still lacking but median survival seems to be longer than 2 years. Most recently, combined ipilimumab and nivolumab was reported in advanced melanoma (6). Here, a response rate of 57% in patients receiving both agents was noted. Importantly, significant activity was also observed in patients with advanced mucosal melanoma (Larkin JJ,

SMR 2015). However, dual immune-checkpoint blockade is accompanied by severe toxicity, which also occurs at a lower rate in monotherapy targeting either CTLA-4 or PD-1. Biomarkers to predict responses to immune-checkpoint blockade could help to avoid such adverse events and identify patients most likely to have a clinical benefit.

The immunological and molecular mechanisms of response or resistance to immune-checkpoint blockade are still poorly understood and thus predictive biomarkers are lacking. In patients receiving CTLA-4 blockade, clinical benefit is associated with a high mutational burden (7,8). This is explained by the fact that somatic mutations give rise to so-called neoantigens. These tumor antigens are recognized by T cells since they are foreign based on the changes in the peptide sequence compared to the wildtype peptide (9). More recently it was shown that non-small cell lung cancer and melanoma patients with a high but also clonal mutational load are more likely to respond to immune checkpoint blockade (10). However, determination of the mutational load is laborious and its association with clinical benefit is too weak to make it a usable biomarker, yet. In PD-1 blockade, mutational load is also associated with clinical benefit in patients with squamous cell lung cancer (11). In patients with advanced melanoma, Roger Lo's group has recently reported the first genetic analyses of response or resistance towards PD-1 blockade (12). In this study, a total of 34 pre-treatment samples (tissue samples n=32, cell lines n=2) underwent whole exome sequencing (WES) and transcriptomes were determined in 27 of these

samples. Additional samples used were obtained early on treatment (WES n=4, transcriptome n=1). Response to PD-1 blockade was defined as non-progressive disease according to irRECIST. No association of total mutational load and response was found, but patients in the top third regarding the number of mutations showed improved overall survival. Interestingly, some genes affected by mutations were enriched in patients responding to PD-1 blockade. In particular, the authors found that *BRCA2* mutations are found more frequently than expected in responders. However, a multivariate analysis was not performed and the data presented indicate that co-occurrence with other mutations enriched in responders is frequent. *BRCA2* is involved in DNA repair and tumors harboring such mutations (n=7) showed a significantly higher mutational load than tumors with wildtype *BRCA2* (n=31). Although this association is intriguing, functional validation of the *BRCA2* mutations is needed. If any of the other mutated genes enriched in responders are also associated with mutational load was not reported. It had been proposed that patients responding to CTLA-4 blockade harbor a certain neoepitope signature (8) which could not be confirmed (7) and was also not found in the current study by Hugo *et al.* (12). Analyzing the transcriptomes, the authors found an increased expression of genes associated with mesenchymal transition, wound healing, angiogenesis, monocyte/macrophage chemotaxis and immune suppression in samples from patients failing to respond to PD-1 blockade. Expression of CD8, PD-L1, PD-L2 CTLA-4 or of cytolytic molecules was not significantly different between responders and non-responders. CD8 infiltration and PD-L1 expression both determined by immunohistochemistry were previously reported to be associated with response to PD-1 blockade (13,14). This discrepancy can be explained by the fact that the spatial localization of PD-L1 and CD8$^+$ T cells seems to be of critical importance and not the general presence (13). Based on the expression patterns found, the investigators next looked into several datasets containing 26 different transcriptomic signatures [referred to as innate anti-PD-1 resistance signature (IPRES)]. IPRES were enriched for mesenchymal transition, angiogenesis and hypoxia and were found in 9 out 13 non-responding but only in one of the responding tumors in the cohort. Of note, some of the 26 signatures indicate that this phenotype can be induced by inhibition of the mitogen-activated protein kinase (MAPK) pathway. MAPK inhibition can induce remarkable regression in advanced melanoma carrying a BRAFV600.

Currently, the best way to clinically sequence MAPKi and immune-checkpoint blockade remains unclear. However, the current finding of IPRES in MAPKi treated tumors and another report by the same group (15) indicate that cross-resistance to immunotherapy in tumors resistant to MAPKi might be a common feature. IPRES were also found significantly more often in melanoma metastases (90/282) than in primary lesions (6/69). In addition, IPRES were also found in other malignancies indicating a general mechanism of disease progression and resistance to therapy. Taken together, Hugo *et al.* provide the first genomic and transcriptomic analysis of response and resistance to PD-1 blockade in metastatic melanoma. Surprisingly, mutational load, expression of cytolytic molecules, PD-L1 and CD8 expression were not associated with response to PD-1 blockade. This might be explained by the small sample size and methological differences compared to previous studies. Since therapy might impact the results obtained, an analysis of only those samples obtained pretreatment would have been important. No recurrent mutations or neoantigens were found. Some genes carrying mutations were enriched in responding tumors. These observations warrant further analyses and functional validations. However, the authors indicate that an immune-resistance signature was associated with non-responsiveness to PD-1 blockade. The so-called IPRES seems to be a common feature in human malignancies and might indicate resistance to PD-1 blockade among different entities. It will be important to evaluate these findings in an independent cohort and to address the role of IPRES in prospective clinical trials.

Recent data suggests that responses to immune-checkpoint blockade are influenced on a multifactorial level. To understand the mechanisms underlying the efficacy of immune-checkpoint blockade will be key to further improve patient outcome. To do so, hypothesis-driven, translational approaches are needed. In such studies, a sufficient cohort size as well as independent validation will be of key importance. Finally, such findings need to be tested in prospective clinical trial to evaluate their potential role in clinical decision making. As long as such evidence is lacking, immune-checkpoint blockade should not be restricted to certain patient subpopulations. However, this will lead new economic challenges for global health systems that need to be faced.

Acknowledgements

Footnote

References

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