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REVIEW ARTICLE

Identification of responders to immune checkpoint therapy: which biomarkers have the highest value?

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Abstract

Evasion of immune recognition by the innate and acquired immune system is a major principle of tumour cells and belongs to the hallmarks of cancer. Immune checkpoint inhibitor-based cancer therapies targeting the co-inhibitory receptors CTLA-4 or PD-1 have received enormous scientific and clinical attention during the last few years, because of promising clinical results observed in the treatment of different cancer entities including melanoma and cutaneous squamous cell carcinoma. However, the enthusiasm about the effects of the immune checkpoint inhibitors is muted as only a subfraction of patients shows a stable clinical response. To predefine the patient cohorts that may benefit from immune checkpoint therapy, rigorous biomarker analyses, which predict the response to these novel therapies, need to be performed. In addition, combination of immune checkpoint therapy with classical DNA-damaging chemotherapy or radiotherapy, which positively affects tumour neo-antigen presentation, appears to be a promising approach in optimizing patients' response. In this review, we briefly summarize important biomarkers for patient stratification and discuss the current limitations of these biomarkers in defining responders vs. non-responders to immune checkpoint therapy.
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Introduction

To direct the patients' immune response in such a way that it triggers the specific elimination of cancer cells is a long-existing dream of clinicians. Actually, during the last decade this dream became true due to the development of specific immune checkpoint inhibitors which target the co-inhibitory receptors PD-1 and CTLA-4 on T cells.[1,2] This success story was recognized by the award of the 2018 Nobel Prize in Physiology and Medicine to James Allison and Tasuku Honjo.

Activation of $CD8^+$, cytotoxic T cells requires the delivery of two simultaneous, activating signals: one signal is delivered by the MHC class I molecule loaded with a non-self-peptide, such as a tumour-specific neo-antigen, that stimulates the T-cell receptor/CD3 complex, and a second co-stimulatory signal triggered by the B7 ligand through engagement of the CD28 co-stimulatory receptor on the cytotoxic T cell. However, in the absence of the B7-CD28 signal or when the B7.1 ligand is bound by the co-inhibitory immune checkpoint CTLA-4 receptor on T cells, the T cell is inactivated, a process termed anergy. In addition, stimulation of the co-inhibitory PD-1 immune checkpoint receptor on T cells by the PD-1 ligand (PD-1L), which was found to be upregulated in cancer cells, similarly results in T-cell anergy. Activation of CTLA-4 and PD-1 inhibitory receptors proved to be one critical mechanism by which cancer cells evade recognition by the host immune system.

In the past years, a set of immune checkpoint inhibitors targeting CTLA-4, such as the antagonistic antibody ipilimumab, has been generated. Similarly, PD-1 antagonists (nivolumab, pembrolizumab, cemiplimab and tremelimumab) have been developed as well as PD-1L antagonistic antibodies (atezolizumab, durvalumab and avelumab) to block the inhibitory checkpoint signal delivered from PD-1L-overexpressing tumour cells. These tools have been successfully used to treat melanoma patients and recently also cutaneous squamous cell carcinoma (cSCC; cemiplimab) patients.[3,4]

Immune checkpoint inhibitors are approved by the FDA for the treatment of a variety of cancer entities including Hodgkin lymphoma, melanoma, non-small cell lung cancer (NSCLC), renal cancer, liver cancer, gastric cancer, colorectal carcinoma, cSCC and urothelial cancer. These agents are used (i) in

monotherapy (e.g. ipilimumab, pembrolizumab or nivolumab for the treatment of unresectable or metastatic melanoma; or cemiplimab for treating metastatic or locally advanced cSCC); (ii) in combination with chemotherapeutic drugs (e.g. pembrolizumab with carboplatin and paclitaxel for treating patients with metastatic squamous NSCLC); (iii) combined with small molecule inhibitors (e.g. pembrolizumab or nivolumab to treat hepatocellular carcinoma patients after initial treatment with the protein kinase inhibitor sorafenib); or (iv) in combination with other immune checkpoint inhibitors (e.g. ipilumumab plus nivolumab to treat patients with advanced renal carcinoma and unresectable or metastatic melanoma).[5–7]

However, despite tumour screening for expression of CTLA-4 and PD-1 on tumour-infiltrating lymphocytes (TIL) or confirmation of PD-1L expression on the tumour cells, the response rate to immune checkpoint therapy is still limited and, dependent on the tumour entity, only a subset of 10–40% (for the single use of antagonistic antibodies to PD-1 and PD-1L) of the patients responded or showed a stable clinical response.[8–10] To increase the response rates of cancer patients to immune checkpoint therapy, improved stratification of the patient cohorts by making use of predictive biomarkers is required. This review aims at briefly summarizing the most important and promising biomarkers to predict the response to cancer treatment with immune checkpoint inhibitors.

Biomarkers predicting the outcome of immune checkpoint therapy

Reliable biomarkers are key to facilitate precise patient stratification, which is essential to predict the clinical response rates to immune checkpoint therapy. A set of different biomarkers has been used to investigate their correlation with cancer patients' response to immune therapy (Fig. 1). First of all, expression levels of the specific molecules targeted with the respective immune checkpoint therapy need to be confirmed by using immunohistochemistry (IHC) staining for PD-1, CTLA-4 or PD-1L from tumour biopsies, respectively. For example, high PD-1L staining correlates relatively well with the response to anti-PD-1L monotherapy across multiple cancer entities including melanoma, NSCLC, renal cell carcinoma, head and neck SCC, colorectal carcinoma, gastric cancer, pancreatic cancer, castration-resistant prostate cancer and metastatic bladder cancer.[9] Accordingly, PD-L1 expression needs to be determined by an FDA-approved IHC test prior to treating patients with stage III NSCLC without EGFR or ALK mutations with pembrolizumab (anti-PD1), and for treating patients with locally advanced or metastatic urothelial carcinoma with atezolizumab (anti-PD-1L).[6,11]

In addition, the gut microbiome[12] and the tumour microenvironment including cancer hypoxia, tumour acidification and metabolic cues clearly influence the response to immune checkpoint therapy, as discussed in recent review articles.[13,14] Here, we want to briefly summarize and discuss a small selection of biomarkers with great potential to predict patients' response to immune therapy.

Immune cell infiltration

In principle, infiltration of the tumour with lymphocytes, in particular $CD8^+$ cytotoxic T cells, could be expected to be a potent predictive marker of the patient's response to immune checkpoint therapy. Accordingly, it has been initially interpreted that high baseline density of TIL, in particular $CD8^+$ T cells, is indicative of an efficient host immune response, which could be used as a key prognostic biomarker for the response to immune checkpoint therapy. Indeed, in a study with pembrolizumab higher baseline TIL correlated with the first response.[15] However, this was not found with the first response, but only after the second round of treatment in a study that used ipilimumab in metastatic melanoma.[16] In addition, further studies revealed

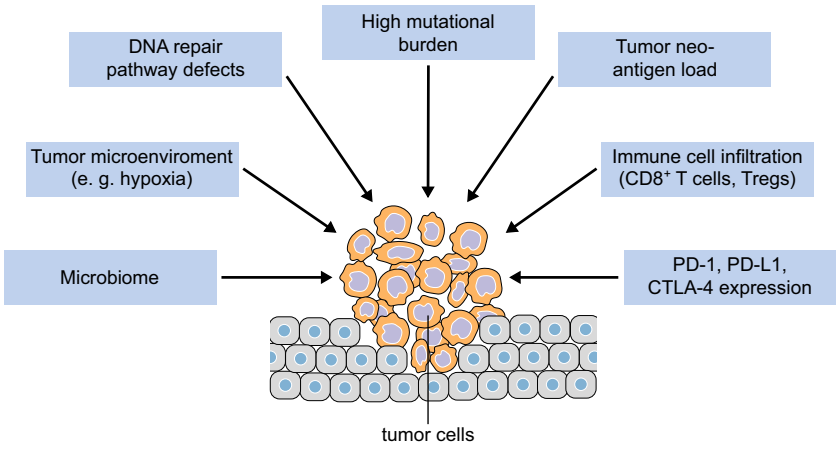

Figure 1 Overview of current biomarkers used to predict the response of cancer patients to immune checkpoint inhibitor therapy.

only moderate correlation[17] between baseline TIL density and immune checkpoint therapy response or even no correlation.[18] These findings suggest that the TIL found in the respective tumour tissues were presumably only activated in some patients. However, in others they stayed inactive or even became anergic. This makes $CD8^+$ TIL densities currently a difficult biomarker with limited predictive power. Thus, more investigations along this biomarker are needed.

The activity of various immune effector cells, including $CD8^+$ cytotoxic T cells, is also controlled by regulatory T cells (T regs), which exert strong immune suppressive functions and induce tolerance.[19] T regs have been found to infiltrate various tumour tissues and are generally regarded as a major immune evasive mechanism in cancer.[19] Thus, it will be important to exactly discriminate between different lymphocyte populations in the tumour biopsies and to specifically determine the density of $FoxP3^+$ T regs in the tumour samples.

DNA repair pathway defects

Cells have a number of repair pathways to maintain genome stability, including homologous recombination (HR) repair and mismatch repair (MMR) during S phase. Functional DNA repair plays a fundamental role in tumour suppression, since cells are continuously confronted with exogenous and endogenous sources of DNA damage.[20] Defective cellular DNA repair pathways lead to accumulation of mutations and increased genomic instability, which are major driving forces of carcinogenesis and a hallmark of cancer. Accordingly, many cancer cells show defective DNA repair pathways, which is also exploited by DNA-damaging chemo- and radiotherapy. For

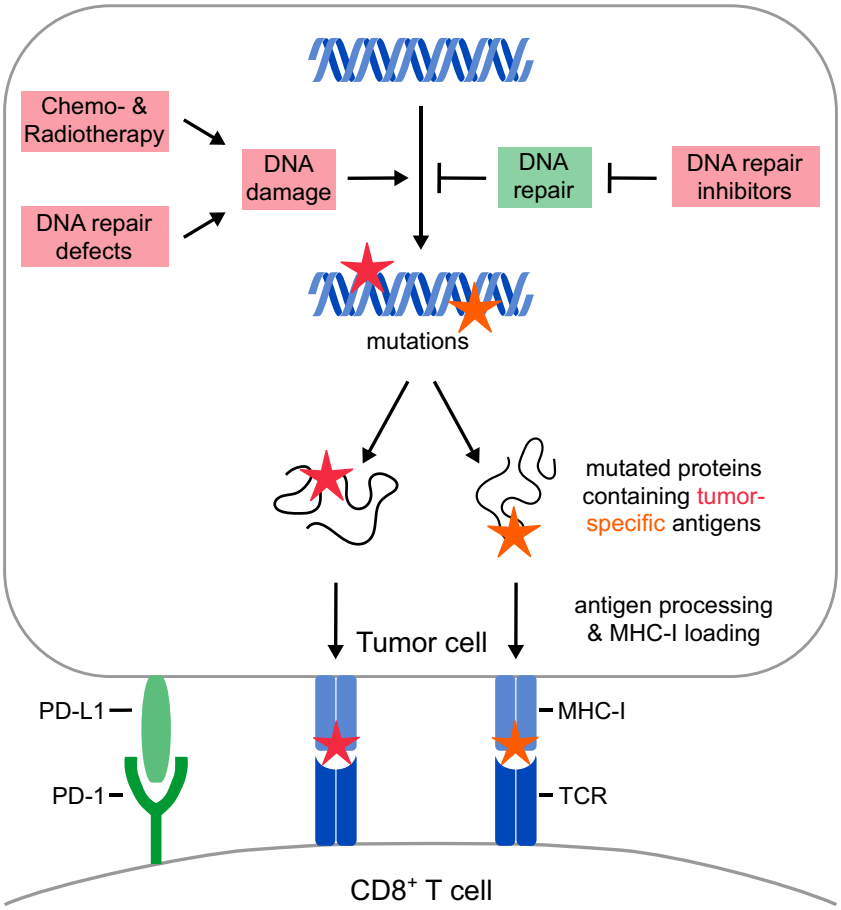

Figure 2 The molecular link between the biomarkers, DNA repair pathway defects, mutational burden and tumour-specific neo-antigen presentation. Cancer cells frequently show DNA repair pathway defects due to mutations or deletions in key DNA repair genes. Defective DNA repair leads to an increase in the mutational burden of the cancer cells. The higher the mutational load, the higher is the likelihood for expression of mutated proteins. After their proteasomal-processing and their subsequent loading on MHC class I molecules, mutated proteins serve as a source of tumour-specific neo-antigens. The MHC I/neo-antigen complex is recognized by specific cytotoxic T cells through their T-cell receptor/CD3 complex and facilitates cancer cell attack and killing.

instance, defective HR (e.g. due to mutations of BRCA1 or BRCA2) is frequently found in ovarian cancer (about 50%), breast cancer (10–40%, dependent on the subtype) and prostate cancer (15–25%).[21] Due to the defects in the HR DNA repair pathway, those cancers are expected to show an increased mutational burden. In addition, cancer cells also frequently show defects in MMR. This pathway operates during DNA replication and counteracts incorporation of wrong deoxynucleotides into the replicating genomes. Several different cancer types were found to show defects in MMR through somatic mutations. For instance, colon cancers show up to 20% of MMR defects, endometrial cancer (up to 20% MMR defective) as well as gastric (15–20% MMR defective) and up to 10% of ovarian cancer, mostly derived from Lynch syndrome patients.[22,23] MMR defects lead to microsatellite instability (MSI), which can be used as a surrogate marker for MMR deficiency. Of note, MSI has been approved as a predictive biomarker for immune checkpoint inhibitor responsiveness by the FDA.[21] Progressed MSI-high unresectable or metastatic solid cancers and progressed colorectal cancers are eligible for treatment with pembrolizumab (anti-PD1).[6] Thus, analysing specific sets of DNA repair genes or a bioinformatic search for specific DNA damage signatures (linked to specific DNA repair defects in the cancer genomes[24]) will be important to evaluate the potential sensitivity to immune checkpoint inhibition. In summary, DNA repair pathway defects are frequently found in cancer and these defects are expected to specifically increase the mutational burden of these cells.

Mutational burden & tumour-specific neo-antigen load

Both the mutational burden as well as the tumour-specific neo-antigen load have been demonstrated to represent predictive biomarkers for immune checkpoint therapy.[25,26] The higher the mutation burden in a tumour entity, the higher is the expected frequency to express mutant proteins which in turn give rise to tumour cell-specific neo-antigens (Fig. 2). Various tumour entities including melanoma, lung cancer and cSCC show high degrees of mutational burden and thus are expected to present numerous tumour-specific neo-antigens, which, in principle, makes them suitable targets for immune checkpoint therapy. Indeed, tumour mutational burden significantly correlated with clinical benefit from immune checkpoint inhibitors in patients with melanoma, NSCLC, urothelial carcinoma and head and neck SCC. However, there was no clear correlation between tumour mutational burden and response rates on a case-by-case basis. For example, patients suffering from renal cell carcinoma, HPV-positive head and neck SCC or melanoma pretreated with the checkpoint inhibitor ipilimumab did not show a correlation between tumour mutational burden and clinical benefit from PD1 pathway inhibition[27] highlighting the need of further research into tumour mutational burden as a predictive biomarker. Strikingly, the absolute number of mutations per megabase of genomic DNA varies massively between these tumour types of up to a factor of 1000.[28] Unfortunately, there are currently no thresholds determined which may help to separate responders from non-responders to immune checkpoint therapy.

Although there is a correlation between the mutational burden of tumours and the positive response of patients to checkpoint therapy, the more critical point is to find out which mutations indeed activate an immune response since they result in presentation of tumour-specific neo-antigens. The binding of the neo-antigen to the MHC class I molecule requires specific properties of the neo-antigen and of the MHC I molecule, which is determined by the HLA subtype. Interestingly, it appears that the immune response is only driven by a small number of neo-antigens and high clonality of these neo-antigens in a given tumour is beneficial for the response to immune checkpoint therapy.[29,30] Along these lines, high heterogeneity of tumour neo-antigens, which is characterized by high subclonal mutations within the tumour mass decreases the response to immune checkpoint inhibitors. Thus, the frequency of a given neo-antigen in the tumour, which is increased in tumours showing high clonality, has an important impact on the outcome of immune checkpoint therapy.

Conclusions

The identification and validation of robust biomarkers are the keys to selecting the patient cohort that benefits most from immune checkpoint inhibitor treatments. Despite the current testing and use of various biomarkers to predefine the patient's response to immune checkpoint therapy (Fig. 1), there is still major optimization required in order to increase the predictive power of these biomarkers. The current limitations in biomarker identification presumably result from the high complexity and individual variations of the tumour cells, the tumour microenvironment and the infiltrating immune cells including cytotoxic T cells and T regs. Both immune cells and tumour cells can release immunomodulatory signals, such as different cytokines, interleukins and interferons, which directly influence immune cell activity and may feed back on neo-antigen presentation and expression of PD-1L on the tumour cell itself. Despite this complex interplay, one promising possibility is to test different combinations of the various biomarkers. This will be a time-consuming and complicated effort, since it is likely that the predictive biomarker combinations will differ between tumour entities, because of the entity-specific tumour microenvironment. Nonetheless, this effort will very likely pay back, since it is expected to improve the identification of responders to immune checkpoint therapy.

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