Tumour mutational burden: a review

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SUMMARY
Immunotherapeutics, like immune checkpoint blockade (ICB), have demonstrated therapeutic efficacy in a variety of human cancers. Even among the tumour types described as responsive, immunotherapy is only efficient in a minority of the patients. To maximise therapeutic benefits, a biomarker to identify ICB-responders is needed. Tumour mutational burden would correlate with the efficacy of immune checkpoint inhibitors. Clinical evidence for TMB as biomarker already exists in metastatic melanoma, non-small cell lung cancer (NSCLC) and renal cell carcinoma (RCC). In this review an update about tumour mutational burden (TMB) is given.

INTRODUCTION
The landscape of cancer treatment has totally changed and besides surgery, radiotherapy, chemotherapy and tyrosine kinase inhibitors (TKIs), immunotherapy has now become the fifth pillar in the fight against cancer. Immunotherapeutics, including immune checkpoint inhibitors (ICB), showed promising results in a subset of patients with different types of solid as well as haematological tumours. Immune checkpoint inhibitors enhance anti-tumour immune response by targeting the adaptive immune response. Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), programmed death-1 (PD-1) and the PD-1 ligand (PD-L1) are the most investigated immune checkpoints. Inhibition of these checkpoints by administration of antibodies will potentially eliminate cancer cells by anti-tumour immune reactivity. Even among the tumour types described as responsive, immunotherapy is efficient only in a minority of the patients. Previous studies reported response rates to PD-1 (nivolumab or pembrolizumab) inhibition of circa 25% in patients with Non-Small Cell Lung Carcinoma (NSCLC), 40% in metastatic melanoma patients and 20% in patients with a Renal Cell Carcinoma (RCC). Otherwise, some PD-L1-negative cancers are good responders.

There was a need for a predictive clinical tool or biomarker for ICB to identify which patients are likely to respond, maximising the therapeutic benefits. Possible predictors as T-cell level, tumour-infiltrating lymphocytes, tumour immune micro-environment and mismatch repair deficiency were previously described but were not optimal. Evaluation of tumour PD-L1 expression level is currently the most used test. Tumours with a higher PD-L1 expression level show a higher response rate after anti-PD-1/PD-L1 therapy comparing with PD-L1 negative tumours. Disadvantages of this biomarker in clinical practice are different detection methods and lack of standardisation. Cancer cells will accumulate somatic mutations during their evolution. Accumulation of more mutations will result in a higher chance of neoantigens recognised by the immune system. Indeed, the best responses to ICB have been seen in metastatic melanoma and NSCLC, tumours with a high tumour mutational burden (TMB). Together with the rapid development of Next-Generation-Sequencing (NGS), Tumour Mutational Burden has become a popular item in the search for a predictive biomarker for immunotherapy response. This review will give an update about Tumour Mutational Burden.

TUMOUR MUTATIONAL BURDEN
When a tumour grows, cancer cells divide. The result is an accumulation of acquired (somatic) mutations in the cancer...
cells. These changes are not detected in normal body cells. Tumour Mutational Burden (TMB) is under investigation as a quantitative biomarker for predicting the response to immunotherapy, especially to immune checkpoints inhibitors. TMB is the measurement of the amount of these somatic cell mutations in the tumour genome expressed by the number of mutations per Megabase. Germline mutations are excluded because our immune system interpret these mutations as normal.

Several studies showed a significant correlation between patients with a high TMB tumour and higher response rates, longer progression-free survival (PFS) or overall survival (OS). For example the CheckMate-227 study, a phase III trial, recently showed significantly longer PFS with first-line combination immunotherapy (nivolumab (anti-PD1) and ipilimumab (anti-CTLA4)) versus standard chemotherapy in NSCLC patients whose tumours have a high TMB (> 10 mutations/Megabase).

**TUMOUR GENOMIC INSTABILITY**

Genomic instability (GI), the acquirement of mutations in a genome at a high frequency, is a hallmark of cancer often associated with poor patient outcome. Beside Tumour Mutational Burden, Microsatellite Instability (MSI) represents the majority of genomic instability in metastatic cancer patients. MSI is the result of a deficiency in DNA Mismatch Repair (MMR) mechanisms and the accumulation of microsatellites, short tandem repeating DNA-sequences. This can lead to the development of cancer, such as colorectal and endometrial neoplasms. MSI-testing can identify the patients who may be good responders to immune checkpoint inhibitors. Since August 2017, the single-agent nivolumab (anti-PD1 agent) is FDA-approved for the treatment of patients with MSI-H (MSI-high) or dMMR (MMR deficient) metastatic colorectal carcinoma (mCRC) who showed progression after treatment with chemotherapeutics. Based on the CheckMate-142 study, the approval of the combination of nivolumab (anti-PD1) and ipilimumab (anti-CTLA4) followed the next year.

What is the extra value of TMB as a biomarker? First of all, despite the fact that MSI was found in many other cancers, the FDA-approval of MSI as an ICB-response biomarker does not apply to other cancers. Second, nearly all MSI-high patients are TMB-high with a significant correlation between both. But an additional cohort of MSI/TMB-high patients may also benefit from ICB. TMB is a more robust predictor and would maybe replace MSI-H status in mCRC.

**THE LINK BETWEEN TUMOUR MUTATIONAL BURDEN AND ICB RESPONSE?**

How can we now link a high tumour mutational load with a good effect on ICB therapy? Cancers with a higher TMB are likely to produce more neo-antigens, presented on dendritic cells. Neo-antigens are recognised by the immune system which induces an anti-tumour response by stimulating the production of (CD8+) T-cells. These cytotoxic T-lymphocytes (CTLs) induce tumour cell lysis by recognising target antigens, bound to HMC I, presented on tumour cell surfaces. Therefore we would expect cancers with a high mutational load to be characterised by strong T-cell responses. However, the correlation between TMB and the effect of ICB has not always been strong. Furthermore, the formation of neo-antigens is necessary, but not the only essential condition for an anti-tumour response. In addition, a recent analysis of genetic alterations that are present in high immuno-active tumours, demonstrated other mechanisms of immune tolerance. This will be an explanation for the cases in which TMB did not predict the treatment efficacy.

**DETECTION METHODS OF TUMOUR MUTATIONAL BURDEN**

For detection of the Tumour Mutational Burden, DNA sequencing is needed. The first studies about tumour mutational burden (e.g. CheckMate-026) all use Whole-Exome-Sequencing for TMB-detection. Unfortunately, WES is a challenging technique. It costs a lot of time and money. Recent studies (e.g. CheckMate-227) focus on Next-Generation Sequencing as TMB-measurement tool. Next-generation sequencing (NGS) is better known as Comprehensive Genomic Profiling by Massively Parallel Sequencing (CGP). Comprehensive Genomic Profiling is available in all academic institutes and has a high sensitivity, accuracy and reproducibility. With the advent of new detection systems, such as Idylla (Biocartis) or nCounter Analysis system (nano string), the detection of TMB is now beginning to decentralise. How does Comprehensive Genomic Profiling works? All coding indels and substitutions, inclusive synonymous mutations are counted, germine mutations are excluded. Mutations are calculated per Megabase or genome area (= 1 million bases).
Studies showed a high correlation between mutations per Megabase in WES versus CGP. In clinical practice, routine detection by NGS/GCP even has better results. In November 2017 the MSK-IMPACT, a NGS-based test developed for use only at Memorial Sloan Kettering Cancer Center (MSKCC) in New York, became FDA approved. Several weeks thereafter, the FDA approved Foundation Medicine’s Foundation-One CDx for widely clinical use. The F1CDx test is the fifth next-generation sequencing-based test for cancer to be approved by FDA, but is the first such test to be reviewed as part of an FDA–CMS initiative designed to speed promising new technologies to market. Beside TMB, the F1CDx samples are also profiled for MSI by assessing indel characteristics at 114 homopolymer repeat loci in/near the targeted gene regions of the F1CDx profile.

For molecular testing (WES or NGS) researchers need to obtain DNA isolated from adequate tissue (FFPE tumour tissue specimens). For patients with advanced disease this is sometimes a challenging procedure. Therefore, techniques to measure tumour mutational burden in blood plasma (bTMB) are under investigation. A recent retrospective analysis of two large RCTs showed that bTMB can identify patients who have clinically significant improvements in progression-free survival from atezolizumab in second-line and higher NSCLC.

TUMOUR MUTATIONAL BURDEN CUT-OFF POINTS

The cut-off points for high versus low TMB vary among several studies who demonstrated the utility of TMB as a biomarker for ICB response. For example, Goodman et al. reviewed charts of 1,638 cancer patients who had undergone NGS and divided the TMB levels into three categories (low: 1-5 mut/Mb, intermediate: 6-19 mut/Mb and high: >20 mut/Mb) based on the Foundation Medicine official reports. On the other hand, in the CheckMate-227 trial, ≥ 10 mutations/Mb is considered as cut-off for high TMB. This value was based on the CheckMate-568 trial, which showed that a TMB ≥10 mut/Mb was associated with enhanced response to nivolumab/ipilimumab regardless of the PD-L1 expression, with ORRs >40%. Buchhalter et al. recently demonstrated that panel size influences confidence intervals, important test parameters and the cut-off values. Panels smaller than 1 Mbp have a threshold below 10 mut/Mb for high TMB. These small panels are not accurate in the classification of tumours with TMB close to the threshold. Among this study, panels between 1.5 to 3 Mbp are ideally for TMB measurement and also have an optimal cost-benefit equilibrium. By contrast, F1CDx determines TMB on a 1.1 Mbp of sequenced DNA.

CONCLUSION

Tumour Mutational Burden is an important independent biomarker for immunotherapy response prediction. TMB is the measurement of the amount of somatic cell mutations in the tumour genome, germline mutations not included. Clinical evidence for the association between high TMB and response to immune checkpoint inhibitors exist already for metastatic melanoma, NSCLC and RCC. Studies for other tumour types are still ongoing.

For detection of TMB, you need DNA sequencing. Whole-Exome-Sequencing was the detection method initially used in studies. This challenging, expensive and time-consuming technique is nowadays replaced by Next-Generation-Sequencing (or CGP). Mutations per Megabase in WES versus NGS have a high correlation. Unfortunately, the cut-off points for high versus low TMB seem unclear. Another issue about molecular testing are tissue problems. For patients with advanced disease it is sometimes difficult to obtain adequate tissue for DNA extraction. A new technique for TMB detection in peripheral blood (bTMB) is under investigation.

KEY MESSAGES FOR CLINICAL PRACTICE

1. There was a need for a predictive clinical tool for immunotherapeutics to identify which patients are likely to respond, maximising the therapeutic benefits.

2. Tumour Mutational Burden is an important biomarker for immunotherapy response prediction.

3. For detection of the Tumour Mutational Burden, you need DNA sequencing. Most described detection tools are Whole-Exome-Sequencing or Next-Generation-Sequencing (Comprehensive Genomic Profiling).

3. Several studies showed a significant correlation between patients with a high TMB tumour and higher response rates, longer PFS or OS.
TMB as biomarker has also its limitations. Therefore, one has to remember that cancer is not a static disease and can gain mutations as it develops. Further investigations are needed for exploring the timing required for TMB measurement and developing tools for dynamic TMB monitoring. A second consequence of the mutation accumulation is that the neo-antigen expression in the tumour would become heterogeneous. This can affect the local anti-tumour response. McGranahan et al. showed that clonal expression of neo-antigens correlates with an increased response to ICB, rather than tumour mutational load. Clonal neo-antigen expression together with high TMB might be a future potent predictive factor for ICB response.

REFERENCES

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