PROTEOME INSTABILITY AS A THERAPEUTIC VULNERABILITY IN MISMATCH REPAIR-DEFICIENT CANCER

Deficient DNA mismatch repair (dMMR) may be caused by germline or somatic mutations in mismatch repair genes (MLH1, MSH2, MSH3, MSH6 and PMS2) or through epigenetic silencing of MLH1.1 dMMR induces a hypermutator phenotype, also known as microsatellite instability (MSI). Next-generation sequencing identifies MSI in 12 cancer types. The highest prevalence is seen in endometrial cancer (31.4%), followed by colorectal cancer (19.7%) and gastric cancer (GC, 19.1%). MSI was related to better prognosis for colorectal cancer and GC. Moreover, the dMMR/MSI hypermutator phenotype is thought to produce large numbers of immunogenic neoantigens that can be recognised by immune cells, leading to the approval of MSI status as a predictive biomarker for colorectal cancer and GC.1,2

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PERIPHERAL T-CELL EXPANSION PREDICTS TUMOUR INFILTRATION AND CLINICAL RESPONSE AFTER IMMUNOTHERAPY

The incredible success of immunotherapy has revolutionised cancer treatment. Nonetheless, more efforts are needed to correctly select patients who will benefit from checkpoint inhibition. Classically, it was presumed that...
immune checkpoint blockade acted on chronically stimulated T cells to reverse their ‘exhausted’ state, like originally observed during chronic virus infection. However, recent observations have questioned this model, suggesting that clinical response could be linked to stem-like memory CD8+ T-cell activity rather than exhausted ones.

Wu et al hypothesised in a recently released paper in Nature that parallel expansion in tumour and normal adjacent tissue (NAT) might represent infiltration of T cells from the periphery, extravasating into both tissues equally from inflamed blood vessels. To prove this hypothesis, they explore and classify profiles of populations of T cells and T-cell receptors (TCRs) in different scenarios: tumorous tissue, NAT and peripheral blood by performing deep single-cell RNA sequencing (scRNA-seq) and TCRs in patients with different tumour types. In an enormous sequencing effort, the authors sequenced more than 330 million mRNA transcripts in 1,416,233 cells from 14 treatment-naïve patients with four different types of cancer.

First, single-cell TCR-sequencing (scTCR-seq) data allowed them to make observations at the level of clones rather than individual T cells. By studying clones, they found that peripheral and intratumorous clone sizes were significantly correlated. This data confirmed that relationship between peripheral expansion and tumour infiltration held not only for aggregate cell fractions but also for individual clones. Second, the authors analysed transcriptional profiles of individual T cells using scRNA-seq, which allowed them grouping of similar cells into clusters. The authors do describe several clusters of T cells not matching published gene signatures, as clusters expressing chromatin remodelling enzymes or apoptosis-related genes. Third, combining scTCR-seq and scRNA-seq discovered more insights into the clonal expansion and behaviour of clones and T cells. Clones of primary CD8 cells were largely dual expanded, whereas clones of CD4+ T cells were generally singletons with exceptions.

They further categorised new tumour clones based on whether they shared TCR sequences with blood samples before treatment in patients. Notably, they found a strong correlation of non-exhausted clones between tumour tissue and blood samples, whereas no correlation was found in exhausted clones. Nevertheless, the authors suggested that the high variability of peripheral clonal expansion and resulting infiltration of T cells in each individual patient could potentially justify differential tumour responses to immune checkpoint blockade. They validated this observation with an extensive evaluation of bulk RNA sequencing tumour samples from three randomised phase II trials of the anti-PD1 (Programmed death Ligand 1 antibody) antibody atezolizumab. Interestingly, a stronger association of progression-free survival with the expression of CCL5, a marker of T-cell activation, is found. This marker was highly expressed in multiple and dual expansion signatures, confirming baseline observations.

In conclusion, it is suggested than non-exhausted T cells and T-cell clones supplied from the periphery may be key factors in explaining patient variability and clinical benefit from cancer immunotherapy. Wu et al considered that clinical benefit from checkpoint blockade could depend directly on non-exhausted T cells that potentially activate an ongoing T-cell response producing a continuous replenishment of tumour-infiltrating lymphocytes. They pointed out the relevant correlation between TCR repertoires of dual-expanded clones in tumours and those of peripherally expanded clones. This close correlation suggests blood may characterise TCR composition of clinically relevant intratumorous T cells. This application could challenge a next revolution in the ‘liquid biopsy’ concept.

WHITE BLOOD CELL AND CELL-FREE DNA (CFDNA) ANALYSES FOR THE DETECTION OF RESIDUAL DISEASE IN RESECTED GC

Despite major breakthroughs in tailored therapy, the survival of patients with GC is still poor. The majority of patients are diagnosed with advanced disease and chemotherapy represents the only possible therapeutic approach. For those patients resected with curative intent, novel non-invasive biomarkers are needed to detect minimal residual disease (MRD) and at higher risk of relapse. Circulating tumour DNA (ctDNA) analysis has demonstrated in many solid tumours to be a relevant tool for detecting MRD after preoperative chemotherapy and after surgery, even when it is undetectable by conventional imaging techniques.

Leal et al recently published in Nature Communications an article that demonstrates how ultrasensitive targeted sequencing analyses of matched ctDNA and white blood cell are able to distinguish ctDNA alterations from genomic aberrations associated with clonal haematopoiesis. This study includes 50 patients recruited in the CRITICS (Chemotherapy versus chemoradiotherapy after surgery and preoperative chemotherapy for resectable gastric cancer) trial, a phase III randomised controlled study of perioperative treatment in patients with resectable GC, assessing the addition of postoperative chemoradiation. For each patient, plasma and buffy coat were collected at baseline, after preoperative chemotherapy and after surgery before initiating adjuvant therapy. After applying the WBC-guided haematopoietic filter, they detected 54 alterations that were likely tumour specific in 27 patients (54%) at baseline. The frequency of mutations according to their panel was TP53, MYC, PIK3CA, RAS, ALK, ATM, KIT and CDH1. Interestingly, they observed that fragments harbouring tumour-specific TP53 mutations were shorter than fragments harbouring TP53 variants arising from clonal haematopoiesis and wild-type sequences (152 bp vs 170 bp). This may thus be another way to differentiate ctDNA alterations from WBC variants. Overall, detection of both WBC and ctDNA variants at baseline did not show statistically significant differences in event-free or overall survival (OS). On the other hand, they evaluated ctDNA measurements before and after neoadjuvant chemotherapy without detecting ctDNA levels in 11 out of 30 evaluable patients after 9 weeks of therapy. In contrast, 19 patients had detectable ctDNA after preoperative treatment and this finding was associated with recurrence after surgery.
After neoadjuvant treatment, seven patients were identified as responders achieving complete or a major pathological response without detectable ctDNA at this timepoint. Lower degrees of pathological response, at least one involved lymph node and detectable ctDNA at this timepoint were related with relapse. They also observed that MRD after surgery from 20 patients with evaluable blood samples at that timepoint predicted recurrence. After a median follow-up of 42 months, 11 out of 20 patients without ctDNA detection at postoperative timepoint were free of relapse. It should be noted that some patients did not recur despite detectable ctDNA after surgery probably due to a potential curative effect of adjuvant therapy. However, the study did not assess ctDNA levels after adjuvant treatment. Detection of ctDNA had a median of 8.9 months lead time over clinical recurrence.

One issue to be taken into account is false-positive rates. Some patients have detectable ctDNA levels in serial plasma samples, harbouring mutations in genes related to clonal haematopoesis. Only when filtering WBC sequence alterations was applied, ctDNA detection after preoperative therapy and curative surgery was significantly associated with higher risk of recurrence, death and shorter OS. In conclusion, this article highlights that sequencing matched cfDNA and WBC detects accurately tumour-specific mutations in cfDNA, without requiring tumour tissue, after neoadjuvant chemotherapy and curative surgery in patients with operable GC. The detection of ctDNA at preoperative and postoperative timepoints was also associated with higher risk of recurrence and shorter median OS.

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