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INTERPLAY OF SOMATIC ALTERATIONS AND IMMUNE INFILTRATION MODULATES RESPONSE TO PD-1 BLOCKADE IN ADVANCED CLEAR CELL RENAL CELL CARCINOMA

Immune checkpoint inhibitors (ICI) have become a key component of therapy for several solid tumours. In patients diagnosed with advanced clear cell renal cell carcinoma (ccRCC), immunotherapy has always been considered as a treatment option, and anti-PD-1-based therapies are approved in both the frontline and refractory settings. Response to PD-1 blockade has been associated with numerous tumour-intrinsic and microenvironment features. Genetic characterisation of ccRCC has significantly contributed to the knowledge of tumour biology and the mechanisms of disease progression, but the interplay of genomic alterations with patterns of immune infiltration in response to PD-1 blockade remains undefined. It is also well known that ccRCC has only a modest mutational burden and a high infiltration of CD8+ T cells.

Recently, a very interesting article has been published in Nature Medicine by Braun et al., trying to answer several questions regarding the relation of molecular alterations and response to ICI in ccRCC. The authors performed an integrated genomic, transcriptomic and immunopathologic analysis of advanced-stage ccRCC tumours derived from 592 patients enrolled in three prospective clinical trials of PD-1 blockade to evaluate the landscape of somatic alterations, copy number changes, the patterns of immune infiltration and the clinical outcome to PD-1 blockade. In this cohort, an integrated genomic and transcriptomic analysis with immune phenotyping by CD8 immunofluorescence was performed showing that CD8+ T-cell infiltrated tumours are relatively depleted for PBRM1 mutations, which correlate with improved survival with anti-PD-1 therapy and are enriched for 9p21.3 deletions, which are associated with worse outcomes after PD-1 blockade.

This integrative approach provides a potential explanation for why CD8+ T-cell infiltration by itself is not associated with response to anti-PD-1 therapy, and also puts forward a conceptual framework for analysing and understanding mechanisms of response and resistance to PD-1 blockade in other tumour types. As commented by the authors, due to the limited number of samples for which immunofluorescence and whole exome sequencing data were available, this potential interplay between CD8+ T-cell infiltration and del9p21.3 will need to be confirmed in future studies of ICI monotherapy in ccRCC. It should be also considered that many patients did receive antiangiogenetic drugs. Therefore, these results cannot be always applied to upfront ICI. Moreover, although it is very impressive, the implementation of the methodology with single-cell analysis and the need for bigger sample size, due the commonly seen heterogeneity, represents a limitation of this analysis. This work, therefore, will be important for ongoing initiatives in precision medicine and immuno-oncology. Moreover, further validation in prospective trials is clearly needed.

THE GENOMIC LANDSCAPE OF INTRINSIC AND ACQUIRED RESISTANCE TO CYCLIN-DEPENDENT KINASE 4/6 INHIBITORS IN PATIENTS WITH HORMONE RECEPTOR POSITIVE METASTATIC BREAST CANCER

The cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) have changed the treatment paradigm of hormone receptor-positive and HER2-negative metastatic breast cancer. These drugs are approved in combination with endocrine therapy and improved progression-free survival and overall survival for treatment-naïve and previously treated patients. Abemaciclib is also approved as monotherapy for pretreated patients. Despite
this initial benefit, many patients present intrinsic and acquired resistance. Thereby, biomarkers with the ability to early identify resistance, or to predict the likelihood of successful treatment using CDK4/6 inhibitors are yet to be identified, and represent an area of unmet clinical need.

Wander et al published in Cancer Discovery an article that performed the genomic landscape of resistance to CDK4/6i via whole exome sequencing of metastatic tumour biopsies. To identify potential mechanisms of resistance, they analysed 59 samples that reflected sensitivity, intrinsic resistance and acquired resistance. They identified eight specific categories of alterations that were enriched in the resistant tumours: biallelic disruption of RB1, activating mutations and/or amplification of AKT1, activating mutations in KRAS/HRAS/NRAS, activating mutations and/or amplification of FGFR2, activating mutations in ERBB2, amplifications of CCNE2, amplification of AURKA and loss of ER (assessed by immunohistochemistry). Interestingly, they showed additional alterations that were not implicated in resistance mechanism of CDK4/6i: TP53 mutations were enriched in resistant specimens, but they were not sufficient to drive resistance; PIK3CA mutations and copy number gains in FGFR1 occurred in both sensitive and resistant samples and ESR1 mutations were related with endocrine resistance.

To confirm, whether these alterations confer resistance to CDK4/6i, they studied two luminal breast cancer cell lines. AKT1, KRAS, AURKA and CCNE2 were overexpressed by lentiviral transduction; and RB1 was suppressed by CRISPR-Cas9. As expected, similar results were obtained at in vitro level, showing that all alterations were sufficient to cause resistance to CDK4/6i. Moreover, they generated acquired CDK4/6i-resistant breast cancer cells to assess whether the drivers identified in patients were also responsible for resistance under selection in vitro. They confirmed that many resistance drivers identified in patient sequencing emerged under selective pressure in vitro. The majority of alterations identified as mechanisms of resistance to CDK4/6 are druggable biomarkers. This opens an opportunity to guide the design of a wide range of precision-based clinical trials, in which patients with specific genomic or molecular alterations are selected to be treated with novel therapeutic combinations aiming at overcoming resistance.

The manuscript shows the first analysis based on whole exome sequencing of sensitive and resistant breast cancer tissues in a cohort of patients who received CDK4/6i. The authors underlined some alterations in several cell cycle regulatory proteins as resistance factors (RB1, CDK6, CCNE1, CCNE2 and AURKA). Moreover, they proposed several oncogenic signalling pathways involved such as ERBB2, FGFR2, AKT1 and RAS, which could be potential targets in novel trial designs.

**REDUCTION OF LIVER METASTASIS STIFFNESS IMPROVES RESPONSE TO BEVACIZUMAB IN METASTATIC COLORECTAL CANCER**

Metastatic colorectal cancer (mCRC) represents a leading cause of cancer-related death worldwide. At diagnosis, 20%–30% of patients suffer from synchronous liver metastases (LM) and 50%–75% of all patients with CRC develop hepatic lesions responsible for the lethality of the disease. Several efforts have been done to better typify mCRC microenvironment to improve the therapeutic approach as it is considered a cause of the primary lack of benefit or resistance to antiangiogenic drugs.

In an interesting paper published on Cancer Cell, Shen et al proposed a deep microenvironment evaluation of primary tumours and LM with the aim of elucidating whether metastatic angiogenesis is affected by the mechanical microenvironment and its relation to antiangiogenic therapy. The authors demonstrated that stiffness of LM in mCRC is higher compared with primary tumours probably because of metastasis-associated fibroblast (MAFs)3 and tissue vascularity. Moreover, the activity of MAFs, and metastasis stiffness, was modulated by commonly used drugs targeting the renin-angiotensin system (RAS). In CRC, LM MAFs were found to express high levels of all RAS components, and RAS inhibition reduces metastases and primary tumour stiffness, attenuating matrix, showing that anti-RAS plus bevacizumab increased vascular integrity in LM. Anti-RAS drugs were found to reduce interstitial fluid pressure and improved drug delivery. In addition, these drugs can also affect other cells within the microenvironment, such as vascular smooth muscle cells, which were not the focus of this study. Of interest, in this analysis, both anti-RAS and antiangiogenesis approaches were shown to enhance the effectiveness of immunotherapy.

Functional experiments with components of extracellular matrix inhibitors supported the role of tumour rigidity and were able to modify tumour progression in vivo. The authors also identified Yes-associated protein (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) as a central hub in metastatic angiogenesis and show that, in the absence of vascular endothelial growth factor (VEGF), stiff matrices still have sufficient potency to activate YAP/TAZ in endothelial cells, suggesting again stiffness as an escape mechanism from bevacizumab treatment.10 In this analysis, there was no survival difference between hypertension over the population within the bevacizumab treatment group, while a significant survival benefit was found over patients with hypertension who received bevacizumab and anti-RAS drugs, in accordance with previous reports.

In conclusion, by using clinical specimens and fresh patient-derived MAFs, the authors have identified a new therapeutic target, MAF-mediated metastatic stiffness, for treating CRC LM. This study also reveals that MAF-mediated matrix stiffening contributes to the development of resistance to VEGF-blocking therapy and commonly used RAS
inhibitors significantly improve the efficacy of bevacizumab. Nevertheless, further investigations are needed.

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