



Revealing anti-PD-1 resistance mechanisms in HCC: A path towards novel combination immunotherapies

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See Article, pages 163–176

Hepatocellular carcinoma (HCC) represents a major health problem, causing more than 700,000 deaths annually worldwide. Patients with HCC are generally diagnosed at advanced stages when they are no longer eligible for curative ablative therapies.^{1–3} Although treatment for advanced HCC has greatly improved over the last 5 years, significantly improving overall survival, there are still many limitations, such as lack of selection of patients that are most likely to respond to each therapy. The current approved systemic therapies for advanced HCC include the multikinase inhibitors sorafenib, regorafenib, lenvatinib, and cabozantinib; the anti-angiogenic antibody ramucirumab (anti-VEGFR2); the immune checkpoint inhibitors (anti-PD-1) nivolumab and pembrolizumab; and the combination therapies atezolizumab (anti-PD-L1) + bevacizumab (anti-VEGFA), recently established as the standard of care, nivolumab + ipilimumab (anti-CTLA4) and durvalumab (anti-PD-L1) + tremelimumab (anti-CTLA4).⁴ Among the newly available therapies for advanced HCC the immune checkpoint inhibitors have provided the best clinical outcomes. Yet, the clinical efficacy of immune-based therapies is still limited and there is an urgent need to improve the stratification of patients that might benefit from these treatment options.⁵ Revealing the different mechanisms that cancer cells evoke to promote resistance against these novel immune-based therapies in HCC will not only address the development of predictive markers but also pave the way for improved combination strategies.

In a recent issue of *Journal of Hepatology*,⁶ Wei and colleagues described a novel mechanism of immune escape and resistance to anti-PD-1 in patients with HCC. The authors demonstrated that the phosphorylation of ZFP64 (upregulated zinc finger protein 64) triggers the transcriptional activation of CSF1 (macrophage colony-stimulating factor) in tumor cells, promoting macrophage recruitment and their polarization towards an immune-suppressive phenotype in the tumor microenvironment. This

suppressive tumor-immune microenvironment was in turn responsible for fostering tolerance to anti-PD-1 therapy. The scope of the work that Wei and colleagues developed highlights the critical relevance of translational studies in which findings in patient samples from retrospective studies are then corroborated in controlled and precise models of the disease, including *in vitro* and *in vivo* studies, to unveil the underlying biology. Starting from a cohort of patients with HCC that respond differently to anti-PD-1 therapy, Wei *et al.* hypothesized that ZFP64 might be playing a role in the susceptibility to anti-PD-1 as they found this protein consistently increased in progressive disease samples compared to those in stable disease or partial responder samples. By in-depth molecular and biochemical characterization, the authors further elucidated protein kinase C alpha (PKC α) as the upstream kinase for ZFP64 phosphorylation and CSF1 as one of the main transcriptional downstream effectors in HCC cells. By targeting both PKC α and CSF1 they demonstrated a potent anti-tumoral effect in combination with anti-PD-1 therapy.

It is very important to keep in mind that modeling human diseases in rodents can sometimes be challenging but nevertheless tremendously instructive when using the appropriate models for each specific question. *In vivo* modeling allows for the study of disease-specific and carcinogenic mechanisms and provides a platform to screen novel therapeutic targets and potential synergies among available treatments. Wei *et al.*'s study included a transgenic mouse model of hepatitis, subcutaneous and orthotopic tumor xenograft models, and several patient-derived xenograft tumors subcutaneously implanted, which together allowed them to identify a previously unknown mechanism of resistance to anti-PD-1 in HCC. It is worth mentioning that the use of subcutaneous models for liver cancer studies is suboptimal, in particular when it comes to studying microenvironmental factors since the tumor microenvironment is heavily influenced by the organ where the tumor grows. These limitations are highly relevant in the field of liver cancer due to the diverse etiology of the underlying liver disease, the consequent high heterogeneity of HCC, and the widely different responses to immunotherapy in patients with HCC. In fact, it has recently been demonstrated that patients with non-alcoholic fatty liver disease/non-alcoholic steatohepatitis develop specific mechanisms of resistance against anti-PD-1 therapy, demonstrating the relevance of accurate animal models that

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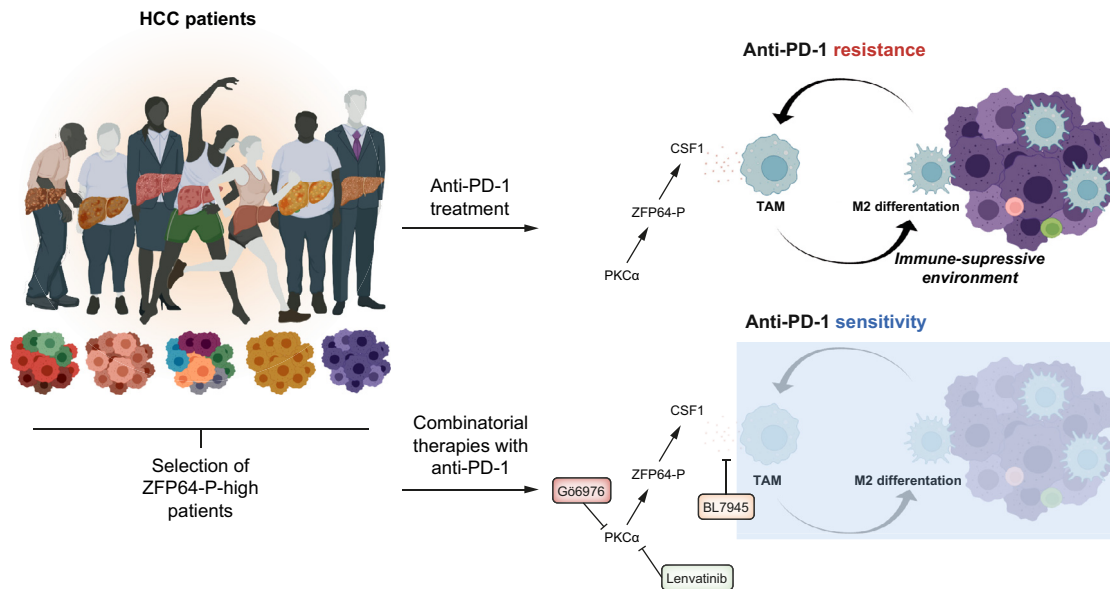


Fig. 1. Selection of ZFP64-P-high HCC patients for combination therapies with anti-PD-1 therapy. HCC, hepatocellular carcinoma.

recapitulate the different aspects of the disease and its heterogeneity.⁷ Nevertheless, the current study by Wei and colleagues elegantly combines multiple mouse models of liver cancer that circumvent important limitations and improve the translational relevance of the findings.

Another key point to address from Wei *et al.*'s study, although not deeply discussed in their manuscript, is the fact that lenvatinib (an approved therapy for HCC) can inhibit the PKC α /ZFP64/CSF1 axis⁶ while sorafenib appears to have no effect on this pathway. The authors demonstrated that lenvatinib, but not sorafenib, specifically improved the tumor-immune microenvironment in HCC mouse models, and further demonstrated the synergistic effects of lenvatinib + anti-PD-1, with similar anti-tumoral effects as the combination of anti-PD-1 with G66976 (inhibitor of PKC α) or BLZ945 (inhibitor of CSF1R). Understanding the underlying mechanisms behind these synergistic effects could be highly relevant for improving clinical practice. The combination of lenvatinib and immune checkpoint inhibitors is being tested as a potential first-line therapy after showing tolerable safety in patients with HCC and very promising prolonged survival rates (phase Ib trial, KEYNOTE-524).⁵ Although further studies will be necessary to understand all the factors implicated in these effects, Wei *et al.*'s study has pointed to a mechanism that can explain the advantage of using lenvatinib vs. other multikinase inhibitors (namely, that it specifically targets the PKC α /ZFP64/CSF1 axis to overcome anti-PD-1 resistance).

Even though this work provides important keys to better understand the different sensitivities to anti-PD-1 in patients with HCC and PKC α /ZFP64/CSF1 pathway upregulation in tumor cells, there are still several highly intriguing unanswered questions. For example, the authors demonstrated through studying patient cohorts and performing *in vitro* studies that the overexpression of this pathway seems to be associated with a high metastatic potential. It would be interesting to address whether activation of this pathway can indeed promote liver tumorigenesis and metastasis, and whether the therapeutic strategies that were tested in the study in primary tumors can also reduce

the metastatic burden. Similarly, it would be of interest to see whether the combination therapy of lenvatinib and anti-PD-1, already showing promising results in an ongoing clinical trial, has an effect in the metastatic capacity of HCC cells in addition to the anti-tumoral effect in primary HCC tumors.

In summary, Wei and colleagues performed a comprehensive study providing new insights into a mechanism of resistance to immunotherapy in HCC mediated by the PKC α /ZFP64/CSF1 axis, pointing towards a potential biomarker for patient stratification in addition to a novel combinatorial therapeutic approach (Fig. 1). In the current context of growing advances in massive sequencing of patient biopsies, along with the development of complex bioinformatic-based *in silico* studies to predict associations between the different patient response groups, it is necessary to highlight the urgent need to validate and understand whether these associations represent a functional biological state in the tumors of patients. In fact, this is a relevant aspect from Wei and colleagues' work, as it provides a blueprint for future studies and exemplifies the conjunction between basic and translational research that could be applied to other diseases and clinical contexts.

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Conflict of interest

The author declares no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure form for further details.

Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhep.2022.04.020>.

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