Immunotherapy for hepatocellular carcinoma: The challenge of biomarker studies

I-Cheng Lee[a,b,*]

[a]*Division of Gastroenterology and Hepatology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, ROC;* [b]*Faculty of Medicine, National Yang-Ming University School of Medicine, Taipei, Taiwan, ROC*

Immune checkpoint inhibitors (ICIs) have altered the treatment landscape for hepatocellular carcinoma (HCC). Anti-programmed cell death-1/programmed cell death-ligand 1 (PD-1/PD-L1) antibody has been approved for second-line or first-line treatment in HCC, including nivolumab, pembrolizumab, and atezolizumab. However, despite the breakthrough in clinical treatment with ICIs, majority of patients do not respond to ICI therapy, especially in the ICI monotherapy setting. Pembrolizumab or nivolumab has an objective response rate of 18%–20% in HCC.[1,2] Therefore, more attentions have been paid to identify predictive biomarkers for the efficacy of ICIs, and more comprehensive understanding has been obtained in recent years, including data on biomarkers of tumor immune microenvironment phenotype, molecular classification, liquid biopsy biomarkers, and host-related factors.[3]

In this issue of the Journal, Hung et al. evaluated PD-1 and PD-L1 expressions on circulating immune cells in patients with HCC before and after Nivolumab monotherapy.[4] Peripheral blood mononuclear cells were collected from 16 patients with advanced HCC undergoing Nivolumab therapy from a phase 1 clinical trial, the author showed that patients with disease control had significantly lower PD-1 expression on B cells, whereas patients with disease progression had increased PD-L1 expression on monocytes after treatment. Patients with disease control tended to show CD8 T cells with lower PD-1 positivity than did patients with disease progression. In general, this study was limited by the small sample size, and these results need to be confirmed by further large prospective studies. Nevertheless, this study provides insights for future research of biomarkers for HCC immunotherapy.

Since the currently available ICIs are targeting the PD-1/PD-L1 pathway, the expressions of PD-1 or PD-L1 on immune cells or tissue are the main research focus to predict efficacy of these ICIs. In some tumor types, such as non-small-cell lung cancer, PD-L1 expression has been associated with improved response.[5] However, the association between PD-L1 expression and efficacy in HCC remains controversial. Initial analyses of CheckMate 040 and KEYNOTE-224 trials did not show a significant correlation between PD-L1 expression and objective response.[2,6] Further analysis of patients in the CheckMate 040 trial showed that tumor PD-1 and PD-L1 expression were associated with improved OS, but complete or partial tumor responses could still be observed in PD-L1-negative patients treated with nivolumab.[7] The PD-1 or PD-L1 expression analysis, which needs tumor tissue sampling, is limited by the invasiveness of the biopsy procedure, sampling error due to intratumor or intertumor heterogeneity, and change tumor microenvironment overtime. In contrast, circulating biomarkers have the advantage of easy sampling and possibility of repeated measurement after treatment, which may make it more convenient for clinical use. For example, circulating PD-1$^+$ CD8 T cells have been shown to correlate with response and disease progression in patients with non-small cell lung cancer treated with immunotherapy.[8] The study by Hung et al also provide promising results of circulating immune cell markers to predict response after ICI therapy for HCC.[4]

A recent study showed that PI3K–mTOR pathway alterations were associated with poorer disease control rate and progression-free survival in sorafenib-treated HCC patients, whereas activating alteration WNT/b-catenin signaling was associated with lower disease control rate, shorter median progression-free survival, and overall survival in HCC patients treated with ICIs.[9] Another study further analyzed these mutational landscapes of advanced HCC using circulating tumor DNA.[10] By performing deep sequencing of 25 targeted genes and Digital Droplet PCR of TERT promoter, the author showed that mutations in the PI3K/mTOR pathway were associated with significantly shorter progression-free survival after tyrosine kinase inhibitors, but WNT pathway mutations were not associated with clinical outcomes after ICI therapy. Therefore, the role of circulating tumor DNA in predicting response to ICI therapy remains to be explored. Recently, several soluble immune checkpoint-related proteins, such as soluble PD-1 and soluble PD-L1, were shown to have promising prognostic value in various cancer types, including HCC.[11] Whether these soluble immune checkpoint-related proteins have prognostic value in HCC patients with ICI therapy worth further study. Recent studies showed that early AFP response could be an on-treatment predictive biomarker of response of ICIs,[12] but currently there is still lack of an ideal baseline circulating biomarker to predict clinical outcomes after ICI therapy for HCC.

Finally, the immune-modulatory effect of loco-regional therapies and targeted therapies may change the immune microenvironment of HCC.[13] Combination of ICI with other novel agents or conventional anti-cancer therapy may further improve the response and survival of advanced HCC, such as atezolizumab plus bevacizumab, and pembrolizumab plus lenvatinib.[14,15] Numerous other immune-modulatory approaches and combinations have been pursued for HCC treatment. A more comprehensive approach to detect key changes in the immune cell

*Address correspondence. Dr. I-Cheng Lee, Division of Gastroenterology and Hepatology, Department of Medicine, Taipei Veterans General Hospital, 201, Section 2, Shi-Pai Road, Taipei 112, Taiwan, ROC. E-mail: iclee@vghtpe.gov.tw (I.-C. Lee).

Conflicts of interest: The author declares that he has no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2021) 84: 121-122.

Received November 3, 2020; accepted November 3, 2020.

doi: 10.1097/JCMA.0000000000000458.

population and tumor microenvironment is needed for future biomarker studies. With the development of research technology such as high-throughput sequencing technology, microarray technology, and artificial intelligence, more and more potential biomarkers could be widely screened on a genomic scale to explore the mechanisms and predictors of immunotherapy efficacy and drug resistance in the future.

REFERENCES

1. Finn RS, Ryoo BY, Merle P, Kudo M, Bouattour M, Lim HY, et al; KEYNOTE-240 investigators. Pembrolizumab as second-line therapy in patients with advanced hepatocellular carcinoma in KEYNOTE-240: a randomized, double-blind, phase III trial. *J Clin Oncol* 2020;**38**:193–202.
2. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase ½ dose escalation and expansion trial. *Lancet* 2017;**389**:2492–502.
3. Bai R, Lv Z, Xu D, Cui J. Predictive biomarkers for cancer immunotherapy with immune checkpoint inhibitors. *Biomark Res* 2020;**8**:34.
4. Hung YP, Shao YY, Lee JM, Hsu C, Hsu CH, Yang MH, et al. Potential of circulating immune cells as biomarkers of nivolumab treatment efficacy for advanced hepatocellular carcinoma. *J Chin Med Assoc* 2021;**84**:144–50.
5. Yan X, Zhang S, Deng Y, Wang P, Hou Q, Xu H. Prognostic factors for checkpoint inhibitor based immunotherapy: an update with new evidences. *Front Pharmacol* 2018;**9**:1050.
6. Zhu AX, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, Verslype C, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. *Lancet Oncol* 2018;**19**:940-52.
7. Sangro B, Melero I, Wadhawan S, Finn RS, Abou-Alfa GK, Cheng AL, et al. Association of inflammatory biomarkers with clinical outcomes in nivolumab-treated patients with advanced hepatocellular carcinoma. *J Hepatol* 2020;**73**:1460–9.
8. Mazzaschi G, Minari R, Zecca A, Cavazzoni A, Ferri V, Mori C, et al. Soluble PD-L1 and circulating CD8+PD-1+ and NK cells enclose a prognostic and predictive immune effector score in immunotherapy treated NSCLC patients. *Lung Cancer* 2020;**148**:1–11.
9. Harding JJ, Nandakumar S, Armenia J, Khalil DN, Albano M, Ly M, et al. Prospective genotyping of hepatocellular carcinoma: clinical implications of next-generation sequencing for matching patients to targeted and immune therapies. *Clin Cancer Res* 2019;**25**:2116–26.
10. von Felden J, Craig AJ, Garcia-Lezana T, Labgaa I, Haber PK, D'Avola D, et al. Mutations in circulating tumor DNA predict primary resistance to systemic therapies in advanced hepatocellular carcinoma. *Oncogene* 2020. Doi: 10.1038/s41388-020-01519-1.
11. Dong MP, Enomoto M, Thuy LTT, Hai H, Hieu VN, Hoang DV, et al. Clinical significance of circulating soluble immune checkpoint proteins in sorafenib-treated patients with advanced hepatocellular carcinoma. *Sci Rep* 2020;**10**:3392.
12. Lee PC, Chao Y, Chen MH, Lan KH, Lee CJ, Lee IC, et al. Predictors of response and survival in immune checkpoint inhibitor-treated unresectable hepatocellular carcinoma. *Cancers (Basel)* 2020;**12**:182.
13. Cheng AL, Hsu C, Chan SL, Choo SP, Kudo M. Challenges of combination therapy with immune checkpoint inhibitors for hepatocellular carcinoma. *J Hepatol* 2020;**72**:307–19.
14. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, et al; IMbrave150 Investigators. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *N Engl J Med* 2020;**382**:1894–905.
15. Finn RS, Ikeda M, Zhu AX, Sung MW, Baron AD, Kudo M, et al. Phase Ib study of lenvatinib plus pembrolizumab in patients with unresectable hepatocellular carcinoma. *J Clin Oncol* 2020;**38**:2960–70.