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Methodological considerations regarding a gene signature to predict microvascular invasion in hepatocellular carcinoma

To the Editor:

We read with great enthusiasm the recent article in *Journal of Hepatology* by Beaufrère *et al.*[1] The authors proposed a 6-gene transcriptomic signatures by using preoperative liver biopsies, which was revealed to be independently associated with the presence of microvascular invasion (MVI) of hepatocellular carcinoma (HCC) by univariate and multivariate logistic regression analyses. Then, the authors demonstrated that this 6-gene signature, as a surrogate maker of MVI, was independently associated with overall survival (OS) after curative resection of HCC by univariate and multivariate Cox-regression analysis. Although interesting, we would like to express the following concerns regarding the statistical analysis in this study:

First, there were many overlapping variables in the multivariate analyses used to predict MVI and OS, as shown in Table 2 and Table S3 in this study.[1] We noticed that the following variables were enrolled into their multivariate analyses, including α-fetoprotein (AFP) score (>2 *vs.* ≤2), Barcelona Clinic Liver Cancer (BCLC) stage (B-C *vs.* 0-A), tumor size (>3 cm *vs.* ≤3 cm), and tumor number (≥2 nodules *vs.* 1 nodule). As we know, the assessment of AFP score requires not only preoperative AFP values but also tumor size and tumor number,[2] while the identification of BCLC stage also requires tumor size and tumor number.[3] That's to say, the variables of tumor size and tumor number were put into the multivariate analyses in the form of 3 overlaps, which inevitably led to fatal errors from the statistical perspective.

Second, recurrence or recurrence-free survival (RFS), but not OS, may be a more appropriate endpoint in this study. It's known that the presence of MVI is closely related to more aggressiveness of HCC, resulting in increased probability of postoperative recurrence following HCC resection.[4,5] Herein, we do not deny the independent association between MVI (or this 6-gene signature as a surrogate marker of MVI) and OS demonstrated by Beaufrère *et al.*, which can be understood as the high mortality due to the high recurrence rate. However, we believe that all readers, like us, are more interested in the results of the correlation between MVI (or the MVI surrogate marker) and recurrence (or RFS). We gratefully appreciate that the authors can present the relevant results for the readers.

Third, the variables related to liver function status were missing in the univariate and multivariate analysis for predicting OS in this study. Numerous studies have shown that variables reflecting preoperative liver function are associated with long-term OS after curative resection for patients with HCC, such as the Child-Pugh grade or the ALBI (albumin-bilirubin) score, *etc.*[6–8] We noticed that Child-Pugh grade appeared in the description of patients' baseline characteristics (Table 1 in this study[1]), but why was this variable not put into the

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univariate and multivariate analyses of OS (Table S3 in this study[1])?

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K-C. W and L-Y. S contributed equally to this letter. K-C. W designed the letter; K-C. W and L-Y. S wrote the letter; M-D. W revised the letter. All the authors reviewed the paper and approved the final version.

Supplementary data
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Author names in bold designate shared co-first authorship

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Reply to: Correspondence regarding "Gene expression signature as a surrogate marker of microvascular invasion on routine hepatocellular carcinoma biopsies"

To the Editor:

We strongly appreciate the interest motivated by our recently published article entitled "Gene expression signature as a surrogate marker of microvascular invasion on routine hepatocellular carcinoma biopsies",[1] and are pleased to consider the different concerns raised by Chao Li *et al*,[2] Zi-Xiang Chen *et al.*[3] and Ke-Chun Wang *et al.*[4] Altogether, they demonstrate that microvascular invasion (MVI), a very major prognostic risk factor, is a challenging issue, and its prediction at the time of hepatocellular carcinoma (HCC) diagnosis remains a significant unmet need.[5,6] This led us to propose an original surrogate 6-gene signature of MVI directly from routine HCC biopsy.

First, as highlighted by Zi-Xiang Chen *et al.*, we fully acknowledge the well-known limits of HCC biopsy (mostly invasiveness and tumor seeding), which are not usually discussed in oncology practice since, as far as we know, tumor biopsy is a prerequisite to treat all patients with cancer, except for HCC. Indeed, the high diagnostic performance of imaging in patients with cirrhosis has pushed the international guidelines to restrict the use of biopsy to atypical cases on imaging.[7] However, a multicentre UK audit showed that the imaging-based diagnostic criteria in advanced HCC had a positive predictive value of 91.4% with differentials that require different patient management.[8] Beyond diagnosis, comprehensive molecular studies have deeply refined the pathological understanding of HCC, with the description of different subgroups associated with different prognostic and theranostic factors.[9] Whereas such information is not yet

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