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LETTER TO THE EDITOR

Prognostic impact of gene copy number instability and tumor mutation burden in patients with resectable gastric cancer

Dear Editor,

Gastric cancer (GC) is a leading cause of cancer-related deaths worldwide, especially in China and other East Asian countries [1, 2]. Although considerable achievements have been made in its treatment [3] and predictive biomarkers [4] in past decades, the prognosis of GC remains poor [5]. Therefore, more effective prognostic markers are needed to improve the prognosis prediction of GCs. Small panels based on next-generation sequencing, such as FoundationOne CDx and MSK-IMPACT, are widely used for selecting appropriate treatment approaches (such as targeted therapies, immunotherapies, and chemotherapies) with the advantages of a higher sequencing depth and more cost-effectiveness than whole-exome sequencing (WES). Previous studies have demonstrated that molecular characteristics based on the designed cancer-related gene panel were consistent with those determined by WES and could be prognostic markers for various cancer types [6-8]. As such, we analyzed the molecular features with the designed panel to investigate probable prognostic biomarkers for Chinese patients with GC.

We selected 100 patients who underwent surgery and histologically diagnosed with GC. Of the 100 patients, 70 were diagnosed at Zhangzhou Affiliated Hospital of Fujian Medical University and classified as the discovery set; 30 were diagnosed at the First People's Hospital of Yunnan Province and were regarded as the validation set. Eighty-nine patients underwent radical gastrectomy, and 11 underwent palliative gastrectomy. Primary and paired adjacent paracancerous tissue samples collected during surgery were used for sequencing. All patients had undergone adjuvant chemotherapy with capecitabine plus oxaliplatin (XELOX). The median number of chemotherapy cycles was 6 (range, 3-12). Overall survival (OS) was defined as the time from surgery to death or the last follow-up. The median follow-up time was 37.3 months (range, 6.0-91.1 months), and 71 patients died during follow-up. The clinical and molecular characteristics of the patients are summarized in Table S1.

To explore the prognostic factors of GC after surgery, univariate and multivariate Cox proportional hazards analyses were performed to examine the association of clinical and molecular features with OS (Table S2). Categorical variables, such as sex, pathological TNM stage, Lauren's classification, degree of histological differentiation, and tumor protein p53 (*TP53)* status were included in the model. Microsatellite instability (MSI) and erb-b2 receptor tyrosine kinase 2 (*ERBB2)* status were excluded because of the extremely small proportions of patients with available data (Table S1). Continuous variables, such as copy number instability (CNI) [9, 10] and tumor mutation burden (TMB) were classified by the optimal cut-off points, which were calculated by X-tile software [11]. The optimal cut-off point was found to be 11,474.1 for CNI and 3.72 mutations/Mb for TMB (data not shown). Age was classified with the median as the cut-off point. $P < 0.05$ was considered significant.

As shown in Table S2, the univariate analysis demonstrated significantly shortened OS when CNI was > 11,474.1 (hazard ratio [HR] = 2.606, 95% confidence interval [CI] = 1.563-4.343, $P < 0.001$) or TMB ≤ 3.72 mutations/Mb (HR = 0.434, 95% CI = 0.241-0.783, $P = 0.006$). The variables with P values < 0.10 were selected for the multivariate analysis. CNI (HR = 2.169, 95% CI = 1.198-3.927; $P = 0.011$) and TMB (HR = 0.475, 95% CI = 0.234-0.965; $P = 0.040$) were identified as independent predictors for OS in GC patients.

Given the importance of clinical and molecular characteristics in cancer prognosis and progression, we also examined the relationships of CNI and TMB with clinical and molecular characteristics. The Fisher's exact test was used to compare these characteristics between the CNI-high and CNI-low groups as well as between the TMB-high and TMB-low groups. Briefly, CNI and TMB were not found to be associated with age, sex, pathological TNM stage,

Abbreviations: CNI, copy number instability; *ERBB2*, erb-b2 receptor tyrosine kinase 2; GC, Gastric cancer; MSI, Microsatellite instability; OS, overall survival; TMB, tumor mutation burden; *TP53*, tumor protein p53; WES, whole-exome sequencing.

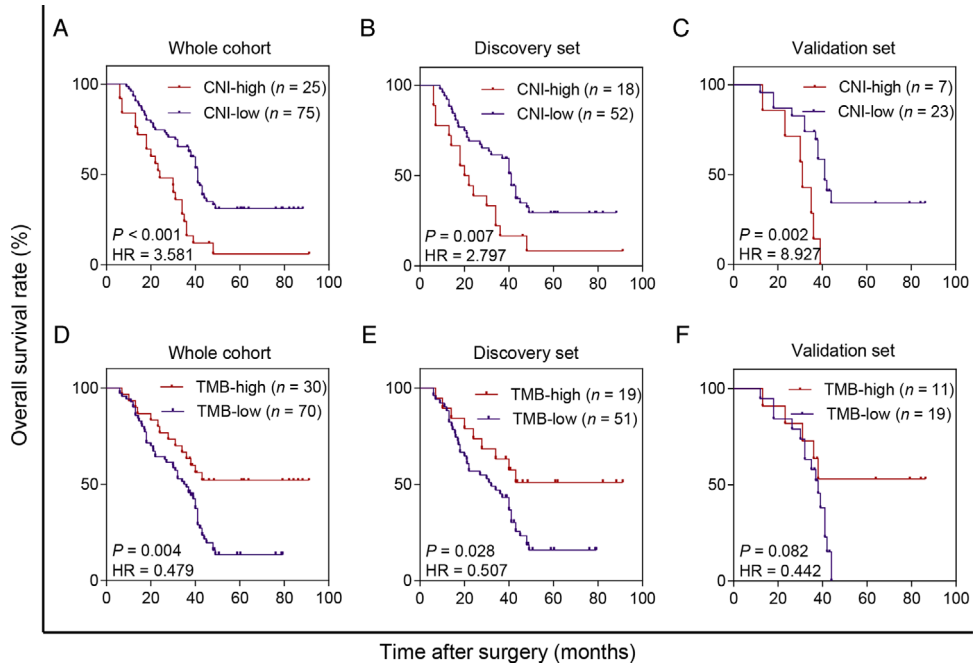

FIGURE 1 Kaplan-Meier overall survival curves of patients with gastric cancer. A patients with high or low CNI in the whole cohort; B patients with high or low CNI in the discovery set; C patients with high or low CNI in the validation set; D patients with high or low TMB in the whole cohort; E patients with high or low TMB in the discovery set; F patients with high or low TMB in the validation set. CNI: copy number instability; TMB: tumor mutation burden

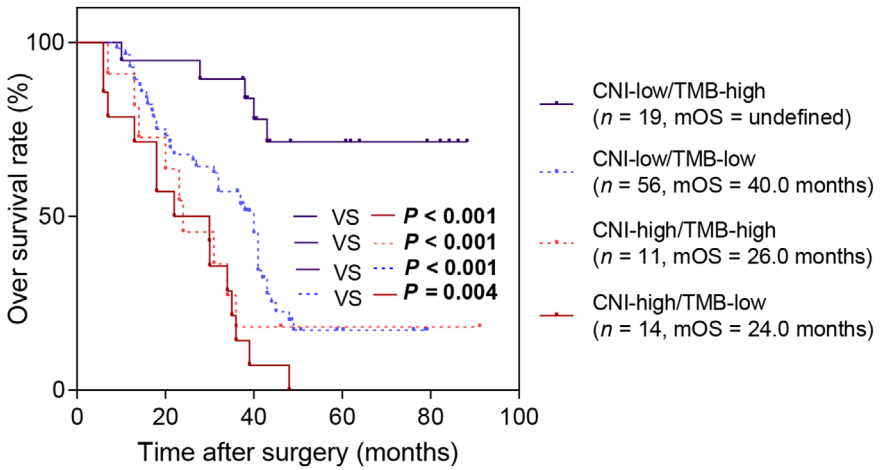

FIGURE 2 Kaplan-Meier overall survival curves illustrate the joint utility of CNI and TMB in predicting the prognosis of gastric cancer. CNI: copy number instability; TMB: tumor mutation burden

Lauren's classification, degree of histological differentiation, or *TP53* status (Table S3).

To further elucidate the prognostic values of CNI and TMB in GC, a Kaplan-Meier survival estimate was used to analyze the associations of CNI and TMB with OS. Low CNI levels were found to be significantly associated with prolonged OS in the whole cohort ($P < 0.001$, Figure 1a) as well as in the discovery set ($P = 0.007$, Figure 1b) and the validation set ($P = 0.002$, Figure 1c). Prolonged OS was associated with high TMB in the whole cohort ($P = 0.004$, Figure 1d) and the discovery set ($P = 0.028$, Figure 1e) but not in the validation set ($P = 0.082$, Figure 1f), probably due to the small proportion of patients with data on MSI-H status. These results indicated that low CNI and high TMB were related to prolonged OS in GC patients and that CNI might be a better prognostic marker than TMB.

Given the prognostic values of CNI and TMB in our study population, we investigated the possibility of CNI combined with TMB in predicting the prognosis for GC patients. The patients were classified into four subgroups based on the estimated X-tile cut-off points for CNI and TMB. Intriguingly, we observed that the CNI-low/TMB-high subgroup had markedly longer median OS than the other three subgroups (log-rank test for trend, $P < 0.001$, Figure 2). Notably, the

median OS was similar in the CNI-high/TMB-high and CNI-high/TMB-low subgroups (26.0 vs. 24.0 months), whereas the median OS of the CNI-low/TMB-low subgroup was significantly longer than that of the CNI-high/TMB-low subgroup (40.0 vs. 24.0 months, $P = 0.004$, Figure 2). The above data suggest that the combination of CNI and TMB could improve patient stratification to strategize postoperative treatment.

Recently, plasma CNI has been increasingly used as a prognostic marker for pancreatic cancer [9] and head and neck squamous cell carcinoma [10]. In accordance with these results, we found that a high CNI could be an independent marker for unfavorable prognosis in patients with resectable GC. TMB has been regarded as a biomarker for predicting the clinical response to immunotherapy and prognosis in various cancer types [12-16]. In the present study, we identified TMB as a prognostic marker for GC patients who had undergone surgery plus adjuvant chemotherapy. In addition, the combination of CNI and TMB might help stratify GC patients with distinct prognosis and should be considered while selecting appropriate adjuvant protocols for these patients.

In summary, our data demonstrated the prognostic values of tumor CNI and TMB in patients with resectable GC. In this context, the combination of tumor CNI and TMB shed light on patient stratification to select appropriate adjuvant treatment protocols.

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AUTHORS' CONTRIBUTIONS
HZ and BH conceived and designed this study. LC and LL contributed to the diagnosis and the recruitment of the patients and follow-up study. DR, XS, HZ, BH and BM analyzed the data and interpreted of results. LC, LL, and DR drafted and revised the manuscript. HZ and BH provided critical comments and suggestions. All authors reviewed and edited the manuscript. All authors read and approved the final manuscript.

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AVAILABILITY OF DATA AND MATERIALS
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
This study was performed in accordance with the ethical standards and the Declaration of Helsinki and according to national and international guidelines. Surgically procured tumor samples from patients were obtained in the Department of General Surgery, Affiliated Hospital of Fujian Medical University and Department of general surgery, The first people' s Hospital of Yunnan Province, The Affiliated Hospital of Kunming University of Science and Technology with informed patients' consent for research purposes.

CONSENT FOR PUBLICATION
Not applicable.

COMPETING INTERESTS
The authors declare that they have no competing interests.

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