NEOPLASMA accepted, ahead of print manuscript
Cite article as https://doi.org/10.4149/neo_2026_260503N138

Running title: Tissue CEA: A better predictor in CRC

Tissue carcinoembryonic antigen as a superior independent prognostic factor for colorectal cancer over serum CEA: a retrospective cohort study

Xin Lu[1,#], Guixiong Zhang[2,#], Yuanqiang Xiao[3,#], Lizhu Wang[4], Xinjian Huang[5,*], Yitai Xiao[1,6,*]

[1]Department of Radiology, Guangdong Provincial People's Hospital; Guangdong Academy of Medical Sciences, Southern Medical University, Guangzhou, Guangdong, China; [2]Department of Nuclear Medicine, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong , China; [3]Department of Radiology, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China; [4]Department of Radiology, The Fifth Affiliated Hospital of Sun Yat-Sen University, Zhuhai, Guangdong, China; [5]Department of Intensive Care Unit, Sun Yat-Sen University Cancer Center; State Key Laboratory of Oncology in South China; Guangdong Provincial Clinical Research Center for Cancer, Guangzhou, Guangdong, China; [6]Department of Endoscopy, Sun Yat-Sen University Cancer Center; State Key Laboratory of Oncology in South China; Guangdong Provincial Clinical Research Center for Cancer, Guangzhou, Guangdong, China

[*]Correspondence: lijjsysucc@21cn.com; xiaoyt@sysuc.org.cn

[#]Contributed equally to this work.

Received May 3, 2026 / Accepted September 8, 2026

We explored the relationships between tissue carcinoembryonic antigen (t-CEA) and serum CEA (s-CEA) and further pursued their association with long-term survival among postoperative colorectal cancer (CRC) patients. All patients underwent determination of s-CEA levels and t-CEA expression. Pearson correlation analysis was performed to quantify the interactions between two variables. Kaplan-Meier survival analysis and time-dependent receiver operating characteristic (ROC) analyses were performed. Prognostic factors were subjected to analysis using multivariable Cox regression to form prognostic nomograms.

The results showed the positive rate of t-CEA overexpression recorded at 90.96%. No significant correlation was shown between the two variables. CRC patients with high t-CEA expression revealed a significantly poorer overall survival (OS) and progression-free survival (PFS). In unadjusted Kaplan-Meier survival analyses, elevated s-CEA levels were associated with significantly poorer PFS ($p = 0.033$) but not OS ($p = 0.077$). Crucially, multivariable Cox proportional hazards regression confirmed that high t-CEA expression, rather than s-CEA, served as a robust independent prognostic factor for both OS (HR = 2.887, $p = 0.017$) and PFS (HR = 2.500, $p = 0.002$). Nomograms comprising t-CEA were constructed with superior utility in predicting the survival probability of CRC patients.

Accepted manuscript

To conclude, our study demonstrates the superior prognostic value of t-CEA as an independent prognostic factor for CRC compared to s-CEA.

Key words: colorectal cancer; carcinoembryonic antigen; prognosis; nomogram

Colorectal cancer (CRC), being among the most prevalent malignancies, holds the third position in terms of cancer incidence and the second position in cancer-related mortality [1]. Along with the proliferation of cancer screening initiatives, the advancements in surgical techniques, and the emergence of innovative therapeutic options, the prognosis for survival in CRC patients has undeniably improved [2]. However, the specter of disease recurrence persists, leading to ongoing challenges in sustaining patient survival rates [3]. The prevailing TNM staging system, endorsed by the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC), stands as the predominant approach employed for evaluating prognosis and overall survival (OS) in CRC [4]. Given the intricate etiology and considerable heterogeneity of CRC, the reliance on gross anatomy-based TNM staging frequently does not facilitate tailored treatment strategies and precise prognostic evaluations [5]. For instance, patients with CRC who exhibit a lower frequency of KRAS mutations are more prone to derive therapeutic benefits from epidermal growth factor receptor (EGFR) antibody therapy [6]. Conversely, patients characterized by microsatellite instability-high (MSI-H) molecular profiles do not experience a significant advantage upon administration of immune checkpoint blockade [7]. Hence, augmenting the incorporation of additional molecular events alongside clinical factors can facilitate the development of more individualized therapeutic approaches and enhance the precision of prognostic assessments.

Originating from the CEACAM5 gene, carcinoembryonic antigen (CEA) is an important member belonging to the immunoglobulin superfamily of cell adhesion molecules (IgCAMs) [8]. CEA is anchored to the membrane of tumor cell through a glycosylphosphatidylinositol linkage and can be released from cancer cells into the blood circulation after treatment with phosphatidylinositol-specific phospholipase C [9]. In CRC, the disruption of normal tissue and the loss of polarization in tumor cells lead to the secretion of CEA into the circulation, manifesting as serum carcinoembryonic antigen (s-CEA). This transformation has positioned s-CEA as an important monitoring index in clinical practice for discerning the occurrence, progression, or

Accepted manuscript

recurrence of CRC, primarily due to its ease of detection [10]. Since its discovery, CEA has gained recognition as a prominent tumor-associated antigen in human CRC [11]. Elevated s-CEA was observed in 76% of patients initially diagnosed with CRC and was believed to correlate with an augmented risk of recurrence and an unfavorable prognosis [12]. Likewise, tissue carcinoembryonic antigen (t-CEA), which serves as the histological origin of s-CEA, was considered to be associated with higher rates of metastasis, recurrence, and lower survival in CRC [13]. Despite their apparent causality, the association between s-CEA levels and t-CEA expression remains a subject of considerable controversy [13].

Until now, only a limited number of studies have examined the correlation between the intensity of t-CEA expression and long-term survival outcomes in CRC patients. Within the scope of this study, we investigated the prevalence of t-CEA and s-CEA in CRC patients and explored the relationships between t-CEA expression and s-CEA level. Moreover, we further pursued the potential association of t-CEA and s-CEA with long-term survival among postoperative CRC patients.

Materials and Methods

Study design and data collection. A total of 166 CRC patients admitted to the Fifth Affiliated Hospital of Sun Yat-sen University from January 2015 to December 2020 were retrospectively reviewed. All patients underwent radical surgical tumor resection and were pathologically confirmed with CRC. Patients lacking essential clinical information and follow-up data were excluded from the study. OS was defined as the duration starting from the date of surgical intervention to the date of death from any cause or the conclusion of the study. The follow-up endpoint of progression-free survival (PFS) was defined as unfavorable tumor progression (recurrence or metastasis) or death from any cause after surgery.

Ethical approval. Approval for this retrospective cohort study was obtained from the Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-Sen University (Approval number: K170-1) with an informed consent waiver. All methods were performed in accordance with the relevant guidelines and regulations of the Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-Sen University

Evaluation of CEA. All enrolled hospitalized patients underwent determination of s-CEA levels in

Accepted manuscript

venous blood samples at initial surgery. A total of 3 mL of peripheral venous blood was collected, followed by centrifugation to separate the serum. The samples were preserved at -20 °C and the s-CEA level was measured by an electrochemiluminescence immunoassay analyzer (F. Hoffmann-La Roche Ltd., Basel, Switzerland) using chemiluminescence method. An elevation was deemed to be present when an s-CEA level exceeded 5 ng/ml.

Immunohistochemistry was employed to evaluate the distribution and expression of t-CEA within CRC tissues. Briefly, the formalin-fixed and paraffin-embedded blocks were cut into 3-μm slices and dewaxed in a 65 °C oven for half an hour. Subsequently, the slices were subjected to deparaffinization using dimethylbenzene and underwent dehydration. After antigen retrieval, the sections were subjected to an overnight incubation in a moist chamber at 4 °C with an anti-CEACAM5 antibody (#ab91213, Abcam, Cambridge, UK) at a dilution of 1:200. Subsequently, the slices were incubated with horseradish peroxidase (HRP)-conjugated rabbit anti-mouse antibody (MaxVision, Fuzhou, China) for 30 min at room temperature. The chromogenic reaction was initiated using the DAB Kit (MaxVision, Fuzhou, China), followed by counterstaining with hematoxylin. All images were captured by a slide scanner system (3D HISTECH, Budapest, Hungary) and quantified by 3D Histech QuantCenter software (3D HISTECH, Budapest, Hungary), with an immunohistochemistry score (H-score) ranging from 0 to 300 according to previous criteria [14]. The H-score was automatically calculated by selecting the three 40× fields of view with the strongest staining intensity in the entire section. In the absence of an established standard, we deemed an H-score exceeding 50 as indicative of overexpression, in alignment with previous literature findings [15].

Statistical analysis. All continuous variables were expressed as mean±standard deviation (SD) for normally distributed data or as median (interquartile range, IQR) for data with a non-normal distribution. Statistical analyses were carried out using R (4.1.1., http://www.R-project.org) in conjunction with relevant packages, SPSS (19.0, IBM, Armonk, USA) and Prism (version 7.0, Graphpad Software, San Diego, USA). For information that conforms to a normal distribution and variance homogeneity, significance testing for differences between two groups was performed using the two-sided Student's *t*-test, whereas differences in the means among three or more groups were assessed using a one-way analysis of variance (ANOVA). In cases where data do not conform to a

Accepted manuscript

normal distribution, statistical differences were assessed using nonparametric tests (Mann-Whitney *U* test or Kruskal-Walli's test). Pearson correlation analysis was performed and a correlation coefficient was obtained to assess the linear association between the two variables. Survival analysis was performed utilizing the Kaplan-Meier method and further assessed with the log-rank test for statistical differences. Receiver operating characteristic (ROC) analyses of prognostic factors and combinations were drawn and area under the curve (AUC) values were obtained for assessing their predictive performance. Prognostic factors were subjected to analysis using multivariable Cox proportional hazards regression analysis to identify independent prognostic factors for overall survival (OS) and progression-free survival (PFS). Univariable Cox proportional hazards regression analyses were first performed to evaluate the associations between clinicopathological variables and survival outcomes. Variables with $p < 0.05$ in the univariable analyses were subsequently included in multivariable Cox proportional hazards regression models to identify independent prognostic factors for OS and PFS. Hazard ratios and 95% confidence intervals were calculated. Nomograms were constructed using significant independent predictors and the predictive accuracy of the nomograms were evaluated using calibration curves and concordance indexes (C-indexes), employing 1000 bootstrapping iterations. Decision curve analysis (DCA) was performed to assess the clinical utility of the nomograms. Statistical significance was defined as *P*-values less than 0.05 (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

Results

General characteristics of the patients. As shown in the flow chart, a total of 166 CRC patients with complete follow-up data were enrolled in the study (Supplementary Figure S1). The baseline characteristics of all patients are shown in Supplementary Table S1. Among the 166 included patients, 99 (59.64%) were male and 67 (40.36%) were female, with a mean age of 65.5 (range, 27-90) years. 79.52% (132/166) were diagnosed with rectal cancer while 20.48% (34/166) with colon cancer. All included patients underwent surgical intervention, and 37 patients (22.29%) received preoperative neoadjuvant chemotherapy. Postoperative pathological examination revealed lymph node metastasis in 42 patients (25.30%). Among these patients, 55.42% (92/166) of patients showed preoperative s-CEA elevation (> 5 ng/ml). Higher s-CEA levels were observed in lymph

Accepted manuscript

node-positive CRC patients (7.68 (IQR 24.29) vs. 5.14 (IQR 10.28), p=0.030, Supplementary Table S1). Apart from this, the s-CEA levels did not exhibit a significant correlation with other clinicopathologic parameters, including age, sex, tumor location, American Joint Committee on Cancer (AJCC) stage, differentiation levels and neoadjuvant chemotherapy ($p > 0.05$, Supplementary Table S1).

T-CEA expression in CRC samples. Representative images of human primary CRC tissue, stained with a CEA antibody, illustrated notable overexpression of t-CEA in CRC specimens (Figure 1A), in comparison to adjacent tissues (H-score, 144.4±57.43 vs. 24.68±12.33, $p < 0.0001$, Figure 1B). The positive rate of t-CEA overexpression was recorded at 90.96% (110/166, Figure 1A), which was significantly higher than the proportion of patients with elevated s-CEA levels (90.96% vs. 55.42%). As indicated in Supplementary Table S1, t-CEA overexpression did not demonstrate any significant correlation with various clinicopathologic parameters, including age, sex, tumor location, AJCC stage, differentiation levels and neoadjuvant chemotherapy ($p > 0.05$). Specifically, there was no statistically significant difference in t-CEA levels between colon cancer and rectal cancer patients (p=0.580). It is noteworthy that higher t-CEA expression was observed in lymph node-positive CRC patients (H-score, 159.68±44.16 vs. 139.23±60.56, p=0.046), implying that t-CEA may play an important role in the prognosis of CRC.

Relationship between s-CEA and t-CEA. The release of t-CEA into the bloodstream, leading to the production of s-CEA, occurs in response to the disruption of normal tissue structure and the loss of polarization in tumor cells [10]. To delve deeper into the connection between tissue t-CEA expression and s-CEA levels, a linear regression analysis was conducted to assess their relationship. Our findings revealed significantly higher t-CEA expression in s-CEA-positive patients than in s-CEA-negative patients (p=0.019, Figure 2A). Additionally, we also compared the s-CEA levels between colon cancer and rectal cancer and found no significant difference (Supplementary Table S1, p=0.957). Nevertheless, linear correlation analysis showed no significant correlation between the two variables (p=0.73, Pearson's r=-0.027, Figure 2B).

Prognostic significance of s-CEA and t-CEA in CRC. In the entire patient cohort, the OS was observed to be 81.0 ± 3.8 months, while the PFS was calculated to be 67.1±3.7 months. Subgroup analyses were conducted by categorizing CRC patients based on both s-CEA concentrations and

Accepted manuscript

t-CEA expression. As Kaplan-Meier survival curves illustrated in Figure 3A and Figure 3B, CRC patients with high t-CEA expression revealed a significantly poorer OS (72.1±5.8 vs. 87.6±4.4 months, p=0.032) and PFS (57.2±5.2 vs. 76.3±4.9 months, p=0.0086) compared to those with lower t-CEA expression. Regarding the effect of s-CEA on the prognosis of CRC, the subset of patients with elevated s-CEA levels exhibited significantly poorer PFS (61.9±4.9 vs. 72.7±5.3 months, p=0.033), while no statistically significant difference was observed in terms of OS (75.7±5.4 vs. 86.9±4.4 months, p=0.077) when compared to the group with lower s-CEA levels (Figures 3C, 3D). Intriguingly, among the 37 patients who received neoadjuvant chemotherapy preoperatively, patients with high t-CEA expression had significantly worse OS (Supplementary Figure S2).

In order to assess the accuracy and reliability of CEA in predicting recurrence or progression of CRC, a receiver operating characteristic (ROC) curve analysis was performed. As depicted in Figure 4, t-CEA demonstrated generally demonstrated higher predictive performance than s-CEA for both OS and PFS, particularly at the 1- and 3-year follow-up time points. For OS, the AUCs of t-CEA were 0.68, 0.66, and 0.45 at 1, 3, and 5 years, respectively, compared with 0.61, 0.59, and 0.44 for s-CEA. Similarly, for PFS, the AUCs of t-CEA were 0.70, 0.61, and 0.50, whereas those of s-CEA were 0.58, 0.54, and 0.42 at the corresponding time points. Dynamic AUC analysis demonstrated that t-CEA maintained superior predictive performance over most of the follow-up period compared with s-CEA, although greater fluctuations were observed at later follow-up because of the limited number of patients remaining at risk (Supplementary Figure S3). Overall, t-CEA exhibited better discriminative performance than s-CEA, particularly for short- and intermediate-term prognosis. At the 5-year follow-up, the predictive performance of both biomarkers declined, likely reflecting the limited number of patients remaining at risk during long-term follow-up.

Multivariable Cox regression analysis of prognostic factors for OS and PFS. To identify independent prognostic factors in postoperative CRC, candidate clinicopathological variables were first evaluated by univariable Cox proportional hazards regression (Supplementary Table S2, Supplementary **Figure S4**). Variables with $p < 0.05$ in univariable analysis were subsequently incorporated into the multivariable Cox regression models. As illustrated in Supplementary Table S2 and Figure 5, high expression of t-CEA remained a robust independent risk factor for both poor

Accepted manuscript

221 OS (HR=2.887, p=0.017) and poor PFS (HR=2.500, p=0.002). Apart from that, positive lymph
222 nodes and advanced disease status (stage III or III) were also poor prognostic factors for
223 postoperative CRC, with HRs of 2.659 (p=0.014) and 4.022 (p=0.002) for OS, 2.089 (p=0.010) and
224 2.297 (p=0.003) for PFS being obtained, respectively. In contrast, s-CEA lost its statistical
225 significance in the multivariable models for both OS (HR=1.855, p=0.142) and PFS (HR=1.274,
226 p=0.390).

227 **Nomogram construction and assessment.** The significant independent predictors for OS and PFS
228 were subjected to form predicting nomograms to predict the 1-, 3-, and 5-year survival probability
229 of OS (Figure 6A) and PFS (Figure 6B) for CRC patients. As demonstrated in the calibration curves,
230 the nomograms exhibited reliable accuracy in estimating the 1-, 3-, and 5-year survival probabilities
231 for both OS (Figure 6C) and PFS (Figure 6D) in CRC patients, yielding C-indexes of 0.796 and
232 0.637, respectively. Furthermore, the DCA was performed to evaluate the clinical utility of the
233 nomograms by comparing them with the traditional TNM staging system. Within the clinically
234 relevant threshold probabilities, the newly constructed nomograms for both 3-year OS (Figure 6E)
235 and PFS (Figure 6F) demonstrated a higher net benefit compared to the traditional TNM staging
236 system alone, indicating superior clinical utility. Similar incremental clinical net benefits of the
237 nomograms were also observed in the long-term 5-year DCA evaluations (Supplementary Figure
238 S5).

239

240 **Discussion**

241 Since its discovery, CEA has been regarded as a compelling tumor marker for CRC , as it is highly
242 expressed almost exclusively in colorectal tumors in the form of high antigenic density, with limited
243 expression in normal tissues [16]. Our findings indicated differential elevation of CEA in CRC
244 patients at both histological and serum levels. Although t-CEA expression and s-CEA levels showed
245 no significant linear correlation, the positivity rate of t-CEA was substantially higher than the
246 proportion of patients with elevated s-CEA. Our results further substantiated that t-CEA expression
247 serves as a significant independent prognostic factor for OS and PFS among CRC patients who
248 have undergone radical resection. While elevated s-CEA showed a univariable association with
249 poorer PFS in unadjusted Kaplan-Meier survival curves, multivariable Cox proportional hazards

Accepted manuscript

250 regression demonstrated that only high expression of t-CEA, rather than s-CEA, served as a robust,
251 independent prognostic predictor for both OS and PFS in postoperative CRC patients.
252 Numerous previous studies have demonstrated the asymmetry of s-CEA concentration to t-CEA
253 expression [13, 17, 18], which corroborated the results of our study. Despite the absence of a
254 significant linear correlation between s-CEA and t-CEA based on linear correlation analysis,
255 noteworthy findings emerged: patients with s-CEA-positive tumors exhibited considerably higher
256 t-CEA expression in comparison to patients with s-CEA-negative tumors. This discrepancy may be
257 attributed, at least in part, to methodological variations in the quantification of t-CEA and s-CEA.
258 Nevertheless, it is widely acknowledged that the expression and distribution of t-CEA within tumor
259 cells, along with its potential to be released into the bloodstream, serve as pivotal factors
260 influencing s-CEA levels [17]. One study confirmed that the localization of t-CEA on the
261 basolateral membrane or stromal tissue exerts a more pronounced impact to a greater elevation in
262 s-CEA levels, compared to t-CEA localized on the luminal surface [19]. Elevated s-CEA levels are
263 frequently observed in patients with CRC who exhibit lymphatic or vascular invasion in the primary
264 tumor tissue [20]. Further comprehensive mechanistic studies are warranted to elucidate the
265 intricate ways in which t-CEA influences s-CEA levels.
266 Another salient aspect worth highlighting pertains to the correlation between CEA and various
267 clinicopathological factors. Our findings indicated a close association between the levels or
268 expressions of both s-CEA and t-CEA with the extent of lymph node metastasis in CRC
269 (Supplementary Table S1). This observation, to some degree, offers an explanation for the impact of
270 CEA on CRC prognosis. Our analysis showed no significant differences in t-CEA or s-CEA levels
271 between colon cancer and rectal cancer patients. These findings indicate that the prognostic value of
272 t-CEA and s-CEA may be consistent across different anatomical sites within the colorectal cancer
273 spectrum. This result suggested that the biological behavior of tumors in the colon and rectum,
274 while distinct in some respects, may not significantly influence these specific biomarkers. In terms
275 of functionality, CEA serves as a cell adhesion molecule mediating recognition and adhesion
276 between tumor cells [21]. Mechanistically, CEA might adopt a pro-invasive phenotype by activating
277 metastatic signaling pathways through interactions with the death receptor 5 (DR5) or the type I
278 transforming growth factor-β receptor 1 (TGF-βR1) [22]. Prior *in vitro* and *in vivo* results have also

Accepted manuscript

279 indicated that CEA could potentially induce malignant cellular transformations by impeding cell
280 differentiation and suppressing cell anoikis, a distinct form of apoptosis instigated by the absence of
281 cellular contact with the extracellular matrix [23]. The potential mechanisms remain a subject of
282 controversy but do not diminish its practical utility. This is a favorable finding as it equips clinicians
283 with the ability to make more precise evaluations of the lymph node status of CRC, which in turn
284 have a direct impact on the selection of subsequent treatment strategies and overall prognosis.
285 Several previous studies have presented conflicting results when it comes to the correlation between
286 t-CEA expression and the differentiation of CRC [13, 17]. Although no correlation between the two
287 was revealed in our results, further research with larger samples is required to confirm and validate
288 these findings.
289 While we have consistently underscored the strengths of t-CEA in prognostic evaluation, it is
290 imperative to acknowledge that s-CEA remains advantageous because it is a cost-effective and
291 readily accessible clinical biomarker [24]. The considerable promise of t-CEA in the realm of
292 tumor-targeted diagnosis and therapy renders the marginal cost of t-CEA detection negligible. CEA
293 holds significant promise as a target for achieving effective tumor imaging and drug delivery, as
294 evidenced by its exploration in various studies [25, 26]. SGM-101, a near-infrared fluorescent tracer
295 targeting t-CEA, has demonstrated its clinical efficacy by enhancing the intraoperative
296 identification of both primary tumors and distant metastatic lesions in CRC, thereby increasing the
297 chances of complete tumor resection [26]. Furthermore, CEA is recognized as a well-established
298 target for the design of chimeric antigen receptor (CAR) T cells, which hold potential for
299 immunotherapy of CRC [25]. These fulfillments can primarily be attributed to the remarkable
300 specificity of CEA, which exhibits elevated expression in CRC tumor tissues while maintaining low
301 expression levels in normal tissues.
302 Despite the valuable insights, it is imperative to acknowledge the limitations in our research that
303 cannot be ignored. The choice of postoperative s-CEA levels as an indicator for analysis may be
304 questionable, which could compromise the effectiveness of s-CEA in prognostic evaluation. Since a
305 large proportion of patients with initially normal s-CEA concentrations still show elevated s-CEA
306 concentrations at the time of recurrence, implying that continuous monitoring of s-CEA may be
307 more rewarding [27]. Although our study focused on t-CEA levels measured in postoperative

Accepted manuscript

surgical specimens, we acknowledge the potential value of assessing t-CEA in preoperative diagnostic biopsies. This approach could allow for the evaluation of t-CEA expression prior to any neoadjuvant therapy and facilitate comparisons of t-CEA levels before and after treatment. While our study highlights the prognostic significance of t-CEA levels, particularly in relation to overall survival, we acknowledge the limitation of not being able to assess the response to adjuvant therapies in detail. Among the 37 patients who received neoadjuvant chemotherapy, high t-CEA levels were associated with poorer overall survival, consistent with previous studies [28, 29]. Several additional prognostic factors, including chemotherapy regimen and molecular characteristics (e.g., BRAF mutation and MSI status), were not incorporated into the present analysis because these data were unavailable or incomplete in this retrospective cohort. Future multicenter prospective studies with more comprehensive molecular and treatment-related data are warranted to further validate and extend these findings. Another limitation of the present study is the relatively modest sample size derived from a single-center retrospective cohort. Although the nomogram demonstrated satisfactory discrimination and calibration after internal validation using 1000 bootstrap resamples, external validation in larger multicenter cohorts is still required before routine clinical application.

In conclusion, elevated t-CEA expression is associated with a poorer prognosis in CRC. There was no direct correlation between t-CEA expression and s-CEA levels. T-CEA demonstrates superior prognostic value as an independent prognostic factor for CRC compared to s-CEA.

Acknowledgements: This work was supported by the National Natural Science Foundation of China (82402404), the Open Program of Hubei Province Key Laboratory of Molecular Imaging (2024fzyx011), the China Postdoctoral Science Foundation (2024M763794) and the Science and Technology Project of Guangdong Province (2026A1515011715).

Supplementary data are available in the online version of the paper.

References

Accepted manuscript

[1] SUNG H, FERLAY J, SIEGEL RL, LAVERSANNE M, SOERJOMATARAM I et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021; 3: 209-249. https://doi.org/10.3322/caac.21660

[2] DEKKER E, TANIS PJ, VLEUGELS J, KASI PM, WALLACE MB. Colorectal cancer. Lancet 2019; 10207: 1467-1480. https://doi.org/10.1016/S0140-6736(19)32319-0

[3] BILLER LH, SCHRAG D. Diagnosis and Treatment of Metastatic Colorectal Cancer: A Review. JAMA 2021; 7: 669-685. https://doi.org/10.1001/jama.2021.0106

[4] AMIN MB, GREENE FL, EDGE SB, COMPTON CC, GERSHENWALD JE et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA Cancer J Clin 2017; 2: 93-99. https://doi.org/10.3322/caac.21388

[5] GANESH K. Optimizing immunotherapy for colorectal cancer. Nat Rev Gastroenterol Hepatol 2022; 2: 93-94. https://doi.org/10.1038/s41575-021-00569-4

[6] DIENSTMANN R, VERMEULEN L, GUINNEY J, KOPETZ S, TEJPAR S et al. Consensus molecular subtypes and the evolution of precision medicine in colorectal cancer. Nat Rev Cancer 2017; 2: 79-92. https://doi.org/10.1038/nrc.2016.126

[7] GANESH K. Optimizing immunotherapy for colorectal cancer. Nat Rev Gastroenterol Hepatol 2022; 2: 93-94. https://doi.org/10.1038/s41575-021-00569-4

[8] KUESPERT K, PILS S, HAUCK CR. CEACAMs: their role in physiology and pathophysiology. Curr Opin Cell Biol 2006; 5: 565-571. https://doi.org/10.1016/j.ceb.2006.08.008

[9] SACK TL, GUM JR, LOW MG, KIM YS. Release of carcinoembryonic antigen from human colon cancer cells by phosphatidylinositol-specific phospholipase C. J Clin Invest 1988; 2: 586-593. https://doi.org/10.1172/JCI113636

[10] HAMMARSTROM S. The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues. Semin Cancer Biol 1999; 2: 67-81. https://doi.org/10.1006/scbi.1998.0119

[11] TIERNAN JP, PERRY SL, VERGHESE ET, WEST NP, YELURI S et al. Carcinoembryonic antigen is the preferred biomarker for in vivo colorectal cancer targeting. Br J Cancer 2013; 3: 662-667. https://doi.org/10.1038/bjc.2012.605

[12] BECERRA AZ, PROBST CP, TEJANI MA, AQUINA CT, GONZALEZ MG et al. Evaluating the Prognostic Role of Elevated Preoperative Carcinoembryonic Antigen Levels in Colon Cancer Patients: Results from the National Cancer Database. Ann Surg Oncol 2016; 5: 1554-1561. https://doi.org/10.1245/s10434-015-5014-1

[13] TONG G, XU W, ZHANG G, LIU J, ZHENG Z et al. The role of tissue and serum carcinoembryonic antigen in stages I to III of colorectal cancer-A retrospective cohort study. Cancer Med 2018; 11: 5327-5338. https://doi.org/10.1002/cam4.1814

[14] XIAO Y, ZHANG G, WANG L, LIANG M. Exploration and validation of a combined immune and metabolism gene signature for prognosis prediction of colorectal cancer. Front Endocrinol (Lausanne) 2022; 1069528. https://doi.org/10.3389/fendo.2022.1069528

[15] AWAD MM, OXNARD GR, JACKMAN DM, SAVUKOSKI DO, HALL D et al. MET Exon 14 Mutations in Non-Small-Cell Lung Cancer Are Associated with Advanced Age and

Accepted manuscript

Stage-Dependent MET Genomic Amplification and c-Met Overexpression. J Clin Oncol 2016; 7: 721-730. https://doi.org/10.1200/JCO.2015.63.4600

[16] GOLD P, FREEDMAN SO. Specific carcinoembryonic antigens of the human digestive system. J Exp Med 1965; 3: 467-481. https://doi.org/10.1084/jem.122.3.467

[17] SAITO G, SADAHIRO S, OKADA K, TANAKA A, SUZUKI T et al. Relation between Carcinoembryonic Antigen Levels in Colon Cancer Tissue and Serum Carcinoembryonic Antigen Levels at Initial Surgery and Recurrence. Oncology 2016; 2: 85-89. https://doi.org/10.1159/000447062

[18] PARK JW, CHANG HJ, KIM BC, YEO HY, KIM DY. Clinical validity of tissue carcinoembryonic antigen expression as ancillary to serum carcinoembryonic antigen concentration in patients curatively resected for colorectal cancer. Colorectal Dis 2013; 9: e503-e511. https://doi.org/10.1111/codi.12304

[19] WAGENER C, MULLER-WALLRAF R, NISSON S, GRONER J, BREUER H. Localization and concentration of carcinoembryonic antigen (CEA) in gastrointestinal tumors: correlation with CEA levels in plasma. J Natl Cancer Inst 1981; 3: 539-547.

[20] ZAMCHECK N, DOOS WG, PRUDENTE R, LURIE BB, GOTTLIEB LS. Prognostic factors in colon carcinoma: correlation of serum carcinoembryonic antigen level and tumor histopathology. Hum Pathol 1975; 1: 31-45. https://doi.org/10.1016/s0046-8177(75)80108-0

[21] EIDELMAN FJ, FUKS A, DEMARTE L, TAHERI M, STANNERS CP. Human carcinoembryonic antigen, an intercellular adhesion molecule, blocks fusion and differentiation of rat myoblasts. J Cell Biol 1993; 2: 467-475. https://doi.org/10.1083/jcb.123.2.467

[22] LI Y, CAO H, JIAO Z, PAKALA SB, SIRIGIRI DN et al. Carcinoembryonic antigen interacts with TGF-beta receptor and inhibits TGF-beta signaling in colorectal cancers. Cancer Res 2010; 20: 8159-8168. https://doi.org/10.1158/0008-5472.CAN-10-1073

[23] CAMACHO-LEAL P, STANNERS CP. The human carcinoembryonic antigen (CEA) GPI anchor mediates anoikis inhibition by inactivation of the intrinsic death pathway. Oncogene 2008; 11: 1545-1553. https://doi.org/10.1038/sj.onc.1210789

[24] GULHATI P, YIN J, PEDERSON L, SCHMOLL HJ, HOFF P et al. Threshold Change in CEA as a Predictor of Non-Progression to First-Line Systemic Therapy in Metastatic Colorectal Cancer Patients with Elevated CEA. J Natl Cancer Inst 2020; 11: 1127-1136. https://doi.org/10.1093/jnci/djaa020

[25] CHASE, KUJAWSKI M, J YP, BROWN C, SHIVELY JE. Tumor regression and immunity in combination therapy with anti-CEA chimeric antigen receptor T cells and anti-CEA-IL2 immunocytokine. Oncoimmunology 2021; 1: 1899469. https://doi.org/10.1080/2162402X.2021.1899469

[26] BOOGERD L, HOOGSTINS C, SCHAAP DP, KUSTERS M, HANDGRAAF H et al. Safety and effectiveness of SGM-101, a fluorescent antibody targeting carcinoembryonic antigen, for intraoperative detection of colorectal cancer: a dose-escalation pilot study. Lancet Gastroenterol Hepatol 2018; 3: 181-191. https://doi.org/10.1016/S2468-1253(17)30395-3

[27] GROSSMANN I, de BOCK GH, MEERSHOEK-KLEIN KW, van de VELDE CJ, WIGGERS T. Carcinoembryonic antigen (CEA) measurement during follow-up for rectal carcinoma is useful even if normal levels exist before surgery. A retrospective study of CEA

Accepted manuscript

values in the TME trial. Eur J Surg Oncol 2007; 2: 183-187. https://doi.org/10.1016/j.ejso.2006.10.035

[28] KIM CH, HUH JW, YEOM SS, KIM HR, KIM YJ. Predictive value of serum and tissue carcinoembryonic antigens for radiologic response and oncologic outcome of rectal cancer. Pathol Res Pract 2020; 3: 152834. https://doi.org/10.1016/j.prp.2020.152834

[29] NAKAMURA Y, SHIDA D, TANABE T, TAKAMIZAWA Y, IMAIZUMI J et al. Prognostic impact of preoperatively elevated and postoperatively normalized carcinoembryonic antigen levels following curative resection of stage I-III rectal cancer. Cancer Med 2020; 2: 653-662. https://doi.org/10.1002/cam4.2758

Figure Legends

Figure 1. A) Representative images of t-CEA expression in tumor tissues derived from CRC patients. The staining intensity is denoted by symbols: +++: strong positive, ++: medium positive, +: weak positive, -: negative. Scale bar: below, 50 μm; above, 200 μm. The bars below represent the proportion of patients with different expression levels. Colors range from light to strong representing different staining intensities. B) Quantification of immunohistochemical results of tumor tissues and paracancerous tissues.

Figure 2. A) T-CEA expression in s-CEA-positive or negative group. B) Linear correlation analysis of t-CEA expression and s-CEA levels. r, correlation coefficient.

Figure 3. A, B) Kaplan-Meier survival analyses comparing overall survival (OS) and progression-free survival (PFS), between CRC patients with low t-CEA expression or high t-CEA expression. C, D) Kaplan-Meier survival analyses comparing OS and PFS between CRC patients with low s-CEA level or high s-CEA level.

Figure 4. A) Time-dependent ROC curves for predicting OS at 1, 3, and 5 years. B) Time-dependent ROC curves for predicting PFS at 1, 3, and 5 years. The AUC for each biomarker is shown within each panel.

Figure 5. Forest plots of multivariable Cox regression analysis for OS (A) and PFS (B) in CRC.

Accepted manuscript

456 LNM, lymph node metastasis; NAC, neoadjuvant chemotherapy.

Accepted manuscript

Fig. 1 Download full resolution image

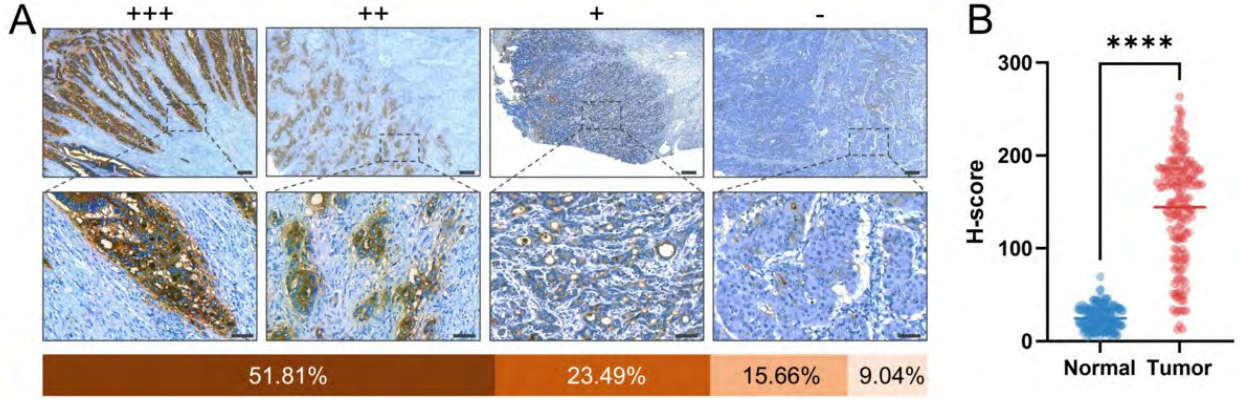

Fig. 2 Download full resolution image

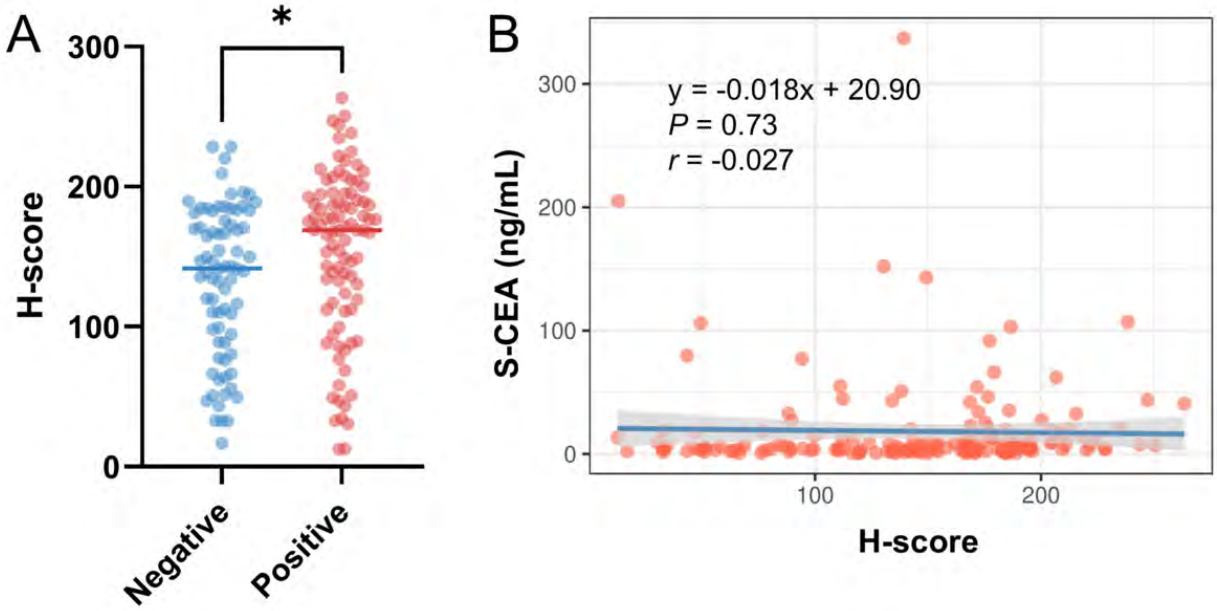

Fig. 3 Download full resolution image

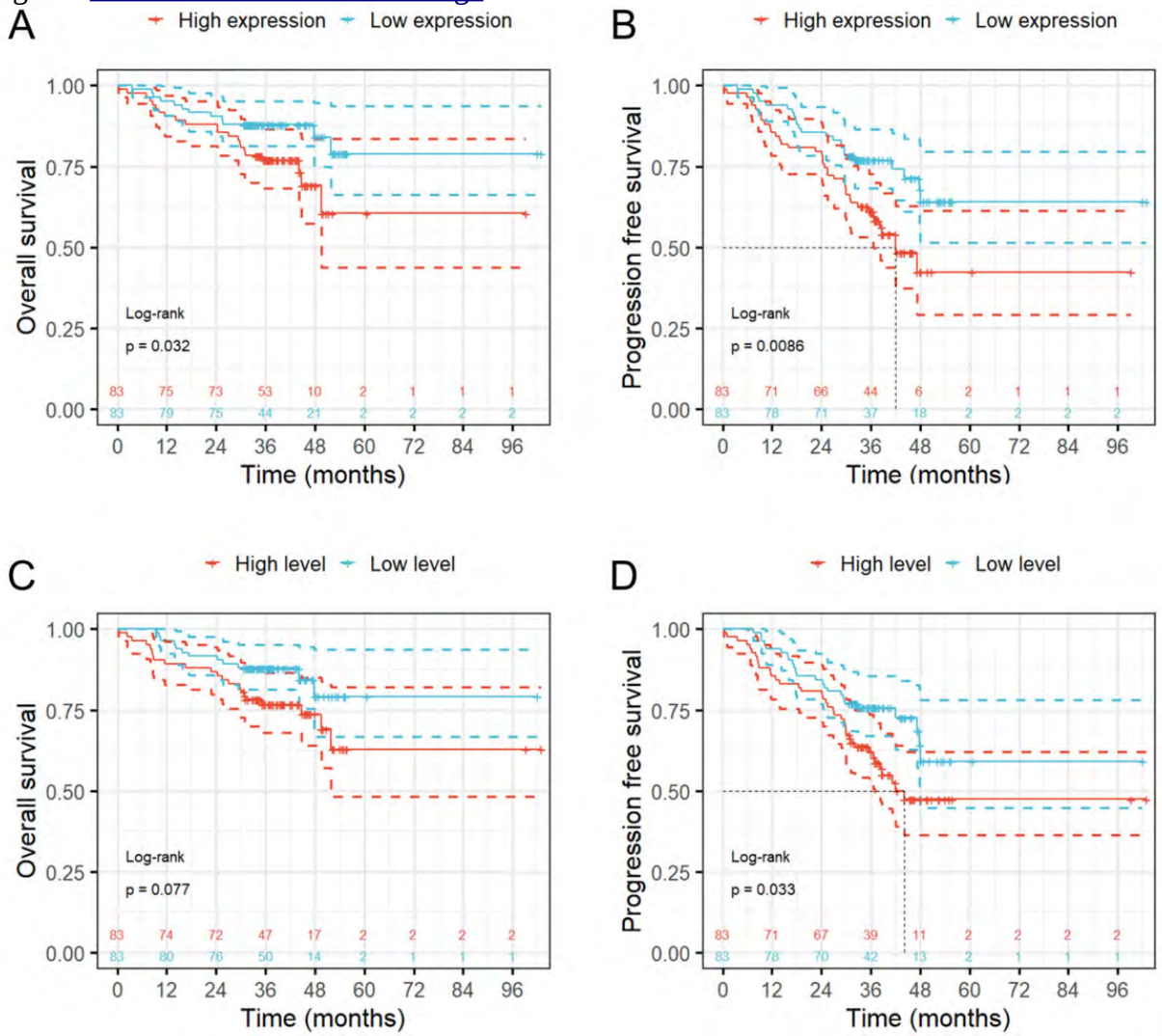

Fig. 4 Download full resolution image

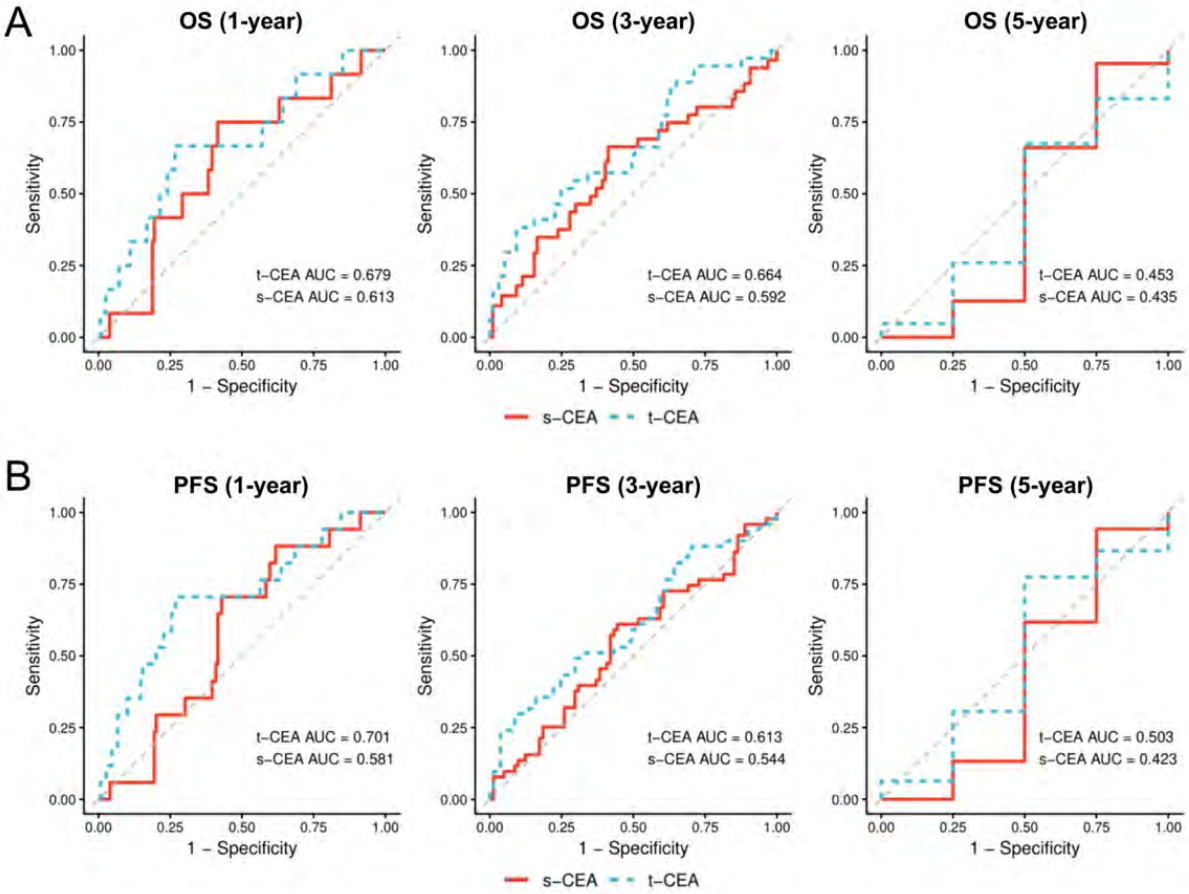

Fig. 5 Download full resolution image

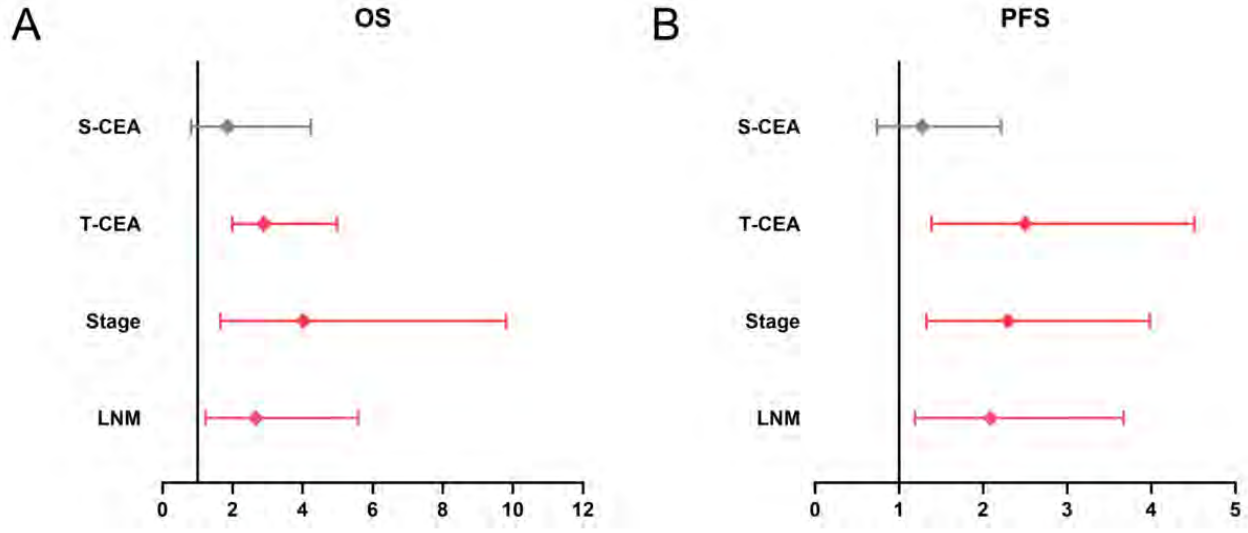
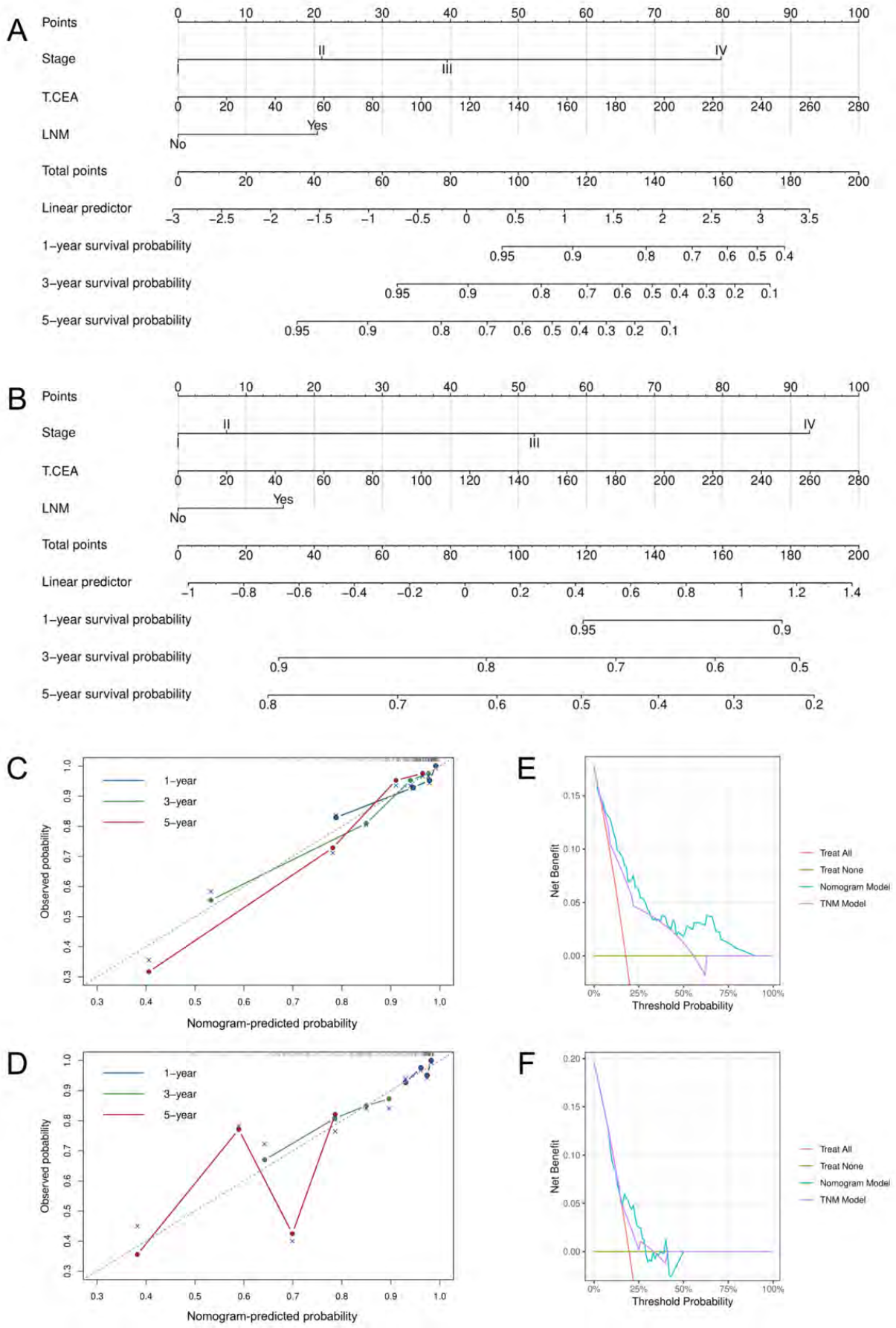
Fig. 6 Download full resolution image