Am J Cancer Res 2026;16(11):4880-4887
www.ajcr.us /ISSN:2156-6976/ajcr0176327

Original Article

Vessel-encapsulating tumor clusters are associated with disease-free survival and lymphovascular invasion in invasive breast cancer: a retrospective single-center cohort study

Hao Chen[1,2,3*], Zhiyuan Zhang[4], Tianyu Zhang[2], Jiquan Liu[2,3], Linjiao Jia[2,3], Shuxin Kong[2,3], Xinyu Zhang[2], Jiaru Gao[2], Jinting Liu[3], Tianxiang Li[5], Sihan Zhang[2], Shicong Cui[6], Yuanxiang Lu[1,2,3*], Wentao Li[1,2,3*]

[1]Department of Breast Surgery, Henan Provincial People's Hospital, Henan Medical University, Zhengzhou 450003, Henan, China; [2]Department of Breast Surgery, Zhengzhou University People's Hospital, Zhengzhou 450003, Henan, China; [3]Department of Breast Surgery, Henan University, Henan Provincial People's Hospital, Zhengzhou 450003, Henan, China; [4]Department of Plastic and Reconstructive Surgery, Southwest Medical University, National Key Clinical Department, Luzhou 646000, Sichuan, China; [5]Department of Dalian Medical University, Dalian 116000, Liaoning, China; [6]Dalian Medical University, Dalian 116000, Liaoning, China. [*]Equal contributors.

Received August 27, 2026; Accepted October 31, 2026; Epub November 15, 2026; Published November 30, 2026

Abstract: This retrospective single-center study examined associations of vessel-encapsulating tumor clusters (VETC) with clinicopathological characteristics and survival in 299 patients with invasive breast cancer. VETC was positive in 141 patients (47.2%) and negative in 158 (52.8%). Lymphovascular invasion (LVI) was present in 91.5% and 40.5% of these groups, respectively ($P < 0.001$). Disease-free survival (DFS) was measured from surgery to first recurrence or death, and overall survival (OS) from surgery to death. Recalculation from recorded dates identified 57 DFS events and 33 deaths. Median follow-up, estimated by reverse Kaplan-Meier analysis, was 40.0 months. VETC-positive patients had shorter DFS (log-rank $P < 0.001$) and OS ($P = 0.025$). The unadjusted hazard ratios (HRs) were 2.88 (95% confidence interval [CI] 1.65-5.03) for DFS and 2.30 (95% CI 1.09-4.84) for OS. After adjustment for LVI alone, the respective HRs were 1.43 (95% CI 0.78-2.62; $P = 0.241$) and 1.57 (95% CI 0.66-3.73; $P = 0.310$). These exploratory findings support associations of VETC with LVI and unadjusted survival outcomes. They do not establish independent prognostic validity, treatment prediction or a causal mechanism.

Keywords: Breast cancer, vessel-encapsulating tumor clusters, CD34, clinicopathological associations, lymphovascular invasion

Introduction

Breast cancer remains the most frequently diagnosed malignancy and a leading cause of cancer-related death among women worldwide. Annual estimates include 2.3 million new cases and 670,000 deaths [1, 2]. Despite advances in systemic therapy and early detection, recurrence and progression remain substantial concerns. Prognostic indicators beyond conventional clinicopathological variables are therefore needed.

The tumor vasculature supplies oxygen and nutrients and provides a route for hematogenous dissemination [3-5]. The arrangement of blood vessels relative to tumor cell nests carries prognostic information that vessel density alone does not capture. Microvessel density, vessel maturation index and peritumoral vascular invasion have been investigated as potential biomarkers across solid tumors [6-11].

Vessel-encapsulating tumor clusters (VETC) were first described in hepatocellular carcinoma as tumor cell clusters completely surrounded by a continuous endothelial network [12, 13]. This architecture facilitates the release of intact tumor clusters into the bloodstream, allowing metastasis without individual cell de-

https://doi.org/10.62347/NEMD9864

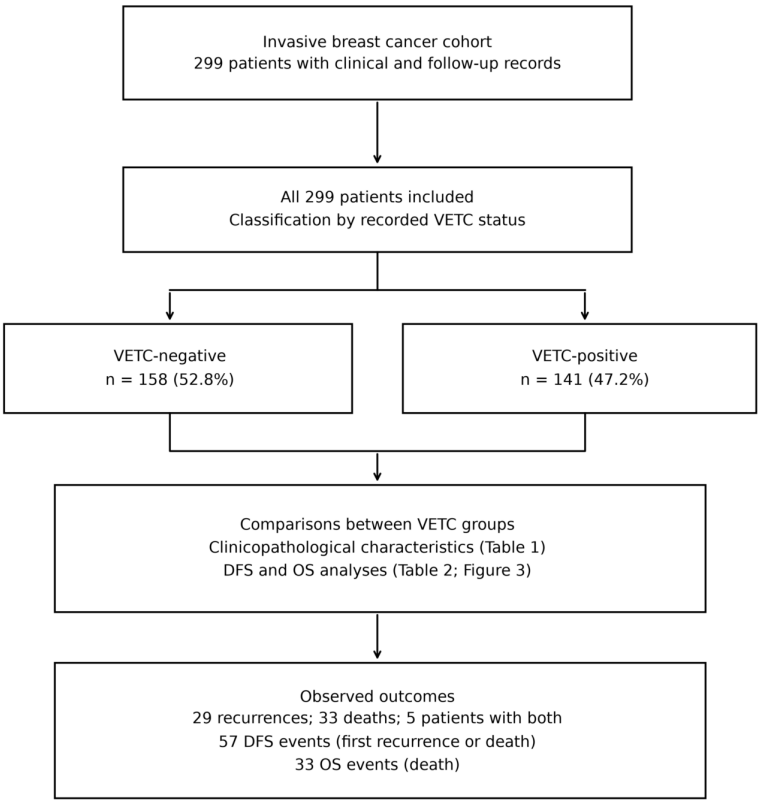


Figure 1. Patient cohort and VETC groups. The cohort comprised 299 patients with invasive breast cancer classified by vessel-encapsulating tumor clusters (VETC). There were 158 VETC-negative and 141 VETC-positive patients. Screening counts could not be reconstructed from the retained clinical records.

tachment. In hepatocellular carcinoma, VETC positivity has been associated with venous invasion, metastatic recurrence and poor overall survival [12, 13].

This study examined associations of VETC with clinicopathological characteristics and survival in a single-center cohort of patients with invasive breast cancer. Histological findings, cell-expression observations and clinical survival analyses were evaluated separately. Cellular expression of cluster of differentiation 34 (CD34) was not interpreted as functional validation of VETC architecture.

Materials and methods

Patient cohort and clinical data collection

This retrospective cohort comprised 299 patients with invasive breast cancer treated at Henan Provincial People's Hospital. Surgery dates ranged from 20 May 2015 to 18 October 2021. The authors confirmed the invasive diagnoses, and anonymous patient identifiers linked the clinical and follow-up records. Histological grade was excluded from clinicopathological comparisons and Cox models because the two recorded grading fields could not be reconciled. Screening counts, consecutive recruitment and the edition used for tumor-node-metastasis (TNM) staging could not be established from the available records. The cohort and VETC groups are summarized in the study flow diagram (**Figure 1**). Ethics approval and informed consent are described in the ethics statement.

Disease-free survival (DFS) was defined as the interval from surgery to first documented recurrence or death from any cause, whichever occurred first. Patients without either event were censored at their last documented recurrence-free assessment. Deaths were counted as DFS events even when they occurred after the last recurrence-free contact. Second primary cancers were not recorded as a separate event category. Overall survival (OS) was defined as the interval from surgery to death from any cause. Survivors were censored at the last date they were confirmed alive. Intervals were calculated in days from medical-record and follow-up dates, with months expressed as days divided by 30.4375. One patient had zero days of follow-up and no event. A sensitivity analysis excluded this patient to assess the effect of this record.

Histological identification of VETC

VETC describes tumor clusters surrounded by an endothelial network [12, 13]. CD34-stained sections illustrate the recorded VETC-negative and VETC-positive classifications (**Figure 2A**). Analyses used this binary classification, and the authors confirmed concordant pathological

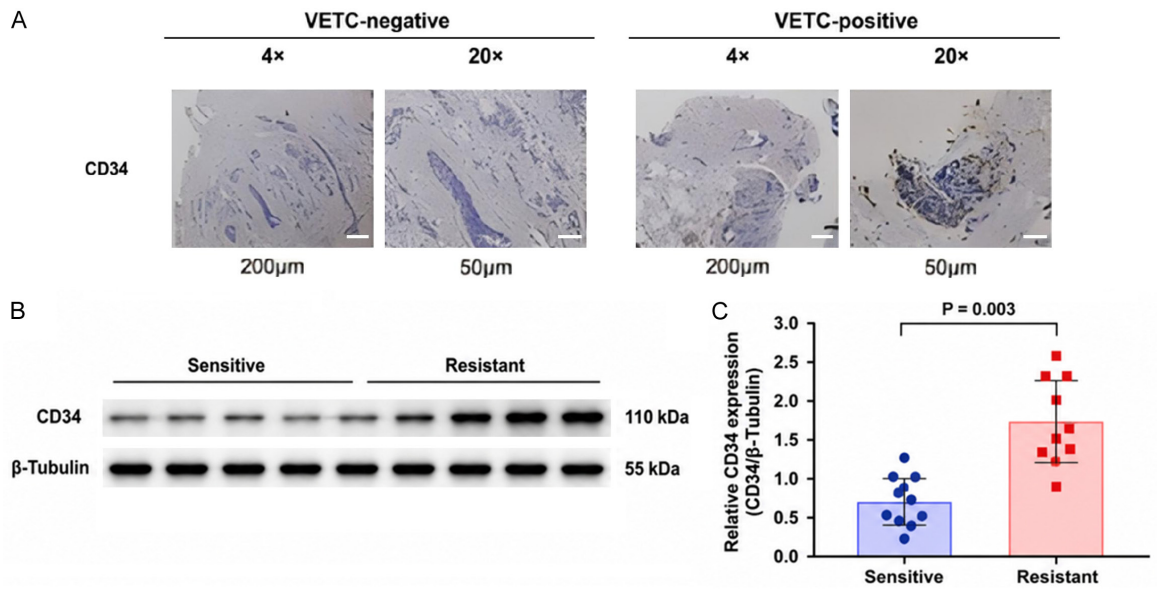


Figure 2. CD34 histological and cell-expression observations. (A) Cluster of differentiation 34 (CD34)-stained histological sections labeled VETC-negative and VETC-positive, shown at the displayed magnifications. (B, C) CD34 immunoblots and expression normalized to beta-tubulin in parental and doxorubicin-resistant breast cancer cells, as described in the original study documentation. Panels (B and C) are descriptive and do not establish VETC architecture or a resistance mechanism. Raw measurements and replicate information were unavailable for verification. The displayed *P* value and error bars are therefore not used for statistical inference. Scale-bar and molecular-weight annotations require confirmation against the original image records.

judgments. Paired independent ratings were not retained, precluding formal assessment of interobserver reliability. Detailed positivity thresholds and field-counting procedures could not be recovered from the scoring records. The quantitative area field was excluded because it was inconsistent with the binary classification.

Assessment of lymphovascular invasion

Lymphovascular invasion (LVI) was analyzed using the binary Recorded LVI field (source code 1, Present; source code 2, Absent). Details of staining, observers and blinding could not be recovered from the original assessment records. This missing information limits assessment of diagnostic reproducibility for this variable.

Ki-67 labeling index assessment

Preoperative Ki-67 category and Postoperative Ki-67 category were analyzed separately. The documented cutoffs were 30% and 20%, respectively, but individual percentage measurements were unavailable to verify their application. Ki-67 interpretation depends on the assessment context [14]. These categorical comparisons were exploratory and were not interpreted as evidence of perioperative biological change. Ki-67 was excluded from all Cox models used in this study.

Descriptive CD34 expression observations

CD34 immunoblots and expression normalized to beta-tubulin are shown for cells described as parental and doxorubicin-resistant breast cancer cells (**Figure 2B**, **2C**). These panels are retained as descriptive observations of the reported expression patterns. Cell-line identity, resistance characterization, independent biological replicate counts, raw densitometry and uncropped blots were unavailable for verification. The displayed statistical annotation was therefore not used as inferential evidence. These observations were not interpreted as validation of VETC architecture or a resistance mechanism.

Scope of exploratory analyses

Single-cell uniform manifold approximation and projection (UMAP) visualizations are included solely as descriptive displays (Figure S1). They do not contribute evidence to the associations

reported in this study. The underlying expression matrices, sample metadata and reproducible analysis outputs were unavailable for verification. Single-cell and public-dataset analyses were therefore excluded from the inferential evidence.

Statistical analysis

Analyses were performed in Python using NumPy, SciPy and lifelines 0.30.3. Age was compared using a two-sided Welch t-test, and categorical variables were compared using Pearson chi-square tests without continuity correction. Available-case denominators were used for each comparison involving missing data. These exploratory comparisons were not adjusted for multiple testing. Survival probabilities were estimated using the Kaplan-Meier method [15] and compared using log-rank tests. Events were processed before censoring when event and censoring times were tied. Median follow-up was estimated by reverse Kaplan-Meier analysis of the OS data. Cox models [16] used Efron handling of ties and included either VETC alone or VETC and LVI in a post hoc two-parameter model. Model convergence, finite estimates and events per coefficient were assessed [17-19]. Proportional hazards were assessed using covariate-specific scaled Schoenfeld residual tests with rank-transformed event times. Kaplan-Meier-transformed times were used in a sensitivity analysis of these tests. These diagnostics were exploratory, and nonsignificant results were not considered proof of proportionality. A sensitivity analysis excluded the patient with zero follow-up. The models were not intended to establish treatment prediction, mediation or independent prognostic validity.

Results

Patient characteristics and clinicopathological associations

All 299 patients were retained, including 141 classified as VETC-positive and 158 as VETC-negative. Mean age was 49.1 years (standard deviation [SD] 10.8), with a between-group *P* value of 0.787. Clinical distributions, denominators and unadjusted comparisons are reported together (**Table 1**).

Recorded LVI was Present in 193 patients, including 129/141 VETC-positive patients and 64/158 VETC-negative patients (91.5% versus 40.5%; $P < 0.001$). The proportion classified as High in the Postoperative Ki-67 category was lower in the VETC-positive group (43/141, 30.5%) than in the VETC-negative group (72/158, 45.6%). The *P* value was 0.007, whereas the Preoperative Ki-67 category comparison yielded $P = 0.756$. The Recorded TNM stage comparison included 298 patients, with one missing value ($P = 0.050$ after rounding). The fluorescence in situ hybridization (FISH) result comparison yielded $P = 0.959$. FISH was not interpreted as a complete characterization of human epidermal growth factor receptor 2 (HER2) status. Histological grade and axillary metastasis were excluded from comparisons because their coding could not be interpreted reliably.

Survival outcomes

There were 29 patients with recurrence and 33 deaths, including five patients who experienced both events. This yielded 57 DFS events, comprising 18 in the VETC-negative group and 39 in the VETC-positive group. VETC positivity was associated with shorter DFS (log-rank $P < 0.001$; **Figure 3A**). The unadjusted hazard ratio (HR) was 2.88 (95% confidence interval [CI] 1.65-5.03; Wald $P < 0.001$). After adjustment for LVI alone, the HR was 1.43 (95% CI 0.78-2.62; $P = 0.241$). Survival estimates from both models are reported alongside the log-rank results (**Table 2**).

There were 33 deaths, comprising 10 in the VETC-negative group and 23 in the VETC-positive group. OS differed between groups defined by VETC status (log-rank $P = 0.025$; **Figure 3B**). The unadjusted HR was 2.30 (95% CI 1.09-4.84; Wald $P = 0.029$). After adjustment for LVI alone, the HR was 1.57 (95% CI 0.66-3.73; $P = 0.310$). Median follow-up was 40.0 months, estimated using reverse Kaplan-Meier analysis. Excluding the zero-follow-up record did not change the log-rank tests or VETC-only Cox estimates. Scaled Schoenfeld tests did not detect departures from proportional hazards. Rank-based *P* values for VETC in the unadjusted DFS and OS models were 0.920 and 0.641, respectively. In the adjusted models, *P* values for VETC and LVI were 0.850 and 0.486 for DFS and 0.608 and 0.648 for OS. Sensitivity tests using Kaplan-Meier-transformed times were also nonsignificant (all $P \geq 0.450$).

Table 1. Clinicopathological characteristics

Recorded field	Total	VETC-	VETC+	*P*
Age mean (SD)	49.1 (10.8)	48.9 (10.7)	49.3 (11.0)	0.787
Menopausal status Premenopausal	112	56	56	0.446
Menopausal status Postmenopausal	187	102	85	
Tumor size ≤ 5 cm	247	129	118	0.642
Tumor size > 5 cm	52	29	23	
Recorded TNM stage I	46	31	15	0.050
Recorded TNM stage II	205	106	99	
Recorded TNM stage III	47	20	27	
Recorded TNM stage missing	1	1	0	
FISH result Negative	240	127	113	0.959
FISH result Positive	59	31	28	
Preoperative Ki-67 category Low	97	50	47	0.756
Preoperative Ki-67 category High	202	108	94	
Recorded LVI Absent	106	94	12	< 0.001
Recorded LVI Present	193	64	129	
Postoperative Ki-67 category Low	184	86	98	0.007
Postoperative Ki-67 category High	115	72	43	
Endocrine therapy No	253	136	117	0.459
Endocrine therapy Yes	46	22	24	
Radiotherapy No	245	123	122	0.095
Radiotherapy Yes	49	31	18	
Radiotherapy missing	5	4	1	
Recorded recurrence No	270	149	121	0.013
Recorded recurrence Yes	29	9	20	
Recorded vital status Alive	266	148	118	0.006
Recorded vital status Deceased	33	10	23	

Group columns follow the recorded VETC classification, and *P* values represent unadjusted comparisons. Denominators vary according to data availability for each recorded field. Histological grade was excluded because the two recorded fields could not be reconciled. Preoperative Ki-67 category and Postoperative Ki-67 category were analyzed as recorded. The FISH result does not provide complete HER2 or molecular-subtype characterization. Recorded recurrence and Recorded vital status report separate components of the composite DFS endpoint. Age was compared using a Welch t-test, and categorical variables were compared using Pearson chi-square tests without continuity correction.

Discussion

VETC was associated with LVI and shorter unadjusted DFS and OS in this invasive breast cancer cohort. Estimates for VETC were attenuated and imprecise after adjustment for LVI alone. These findings neither establish independent prognostic validity nor demonstrate that LVI mediates the observed associations. The histological and cell-expression observations likewise do not establish a causal mechanism of chemoresistance.

Studies of endothelial architecture and tumor stroma provide a biological rationale for investigating VETC [12, 13, 20]. Breast cancer molecular heterogeneity [21] and differences between lymphatic and vascular involvement [22, 23] provide relevant context for interpreting these pathological associations. However, these concepts do not demonstrate that VETC precedes LVI or mediates dissemination in this dataset. Their co-occurrence does not establish the direction or mechanism of the relationship.

The adjusted VETC CIs encompass both no association and potentially meaningful effects. A nonsignificant adjusted result therefore does not exclude a prognostic association. Although residual-based tests did not detect departures from proportional hazards, adjustment for LVI alone leaves potential confounding by treatment, stage, grade and molecular subtype.

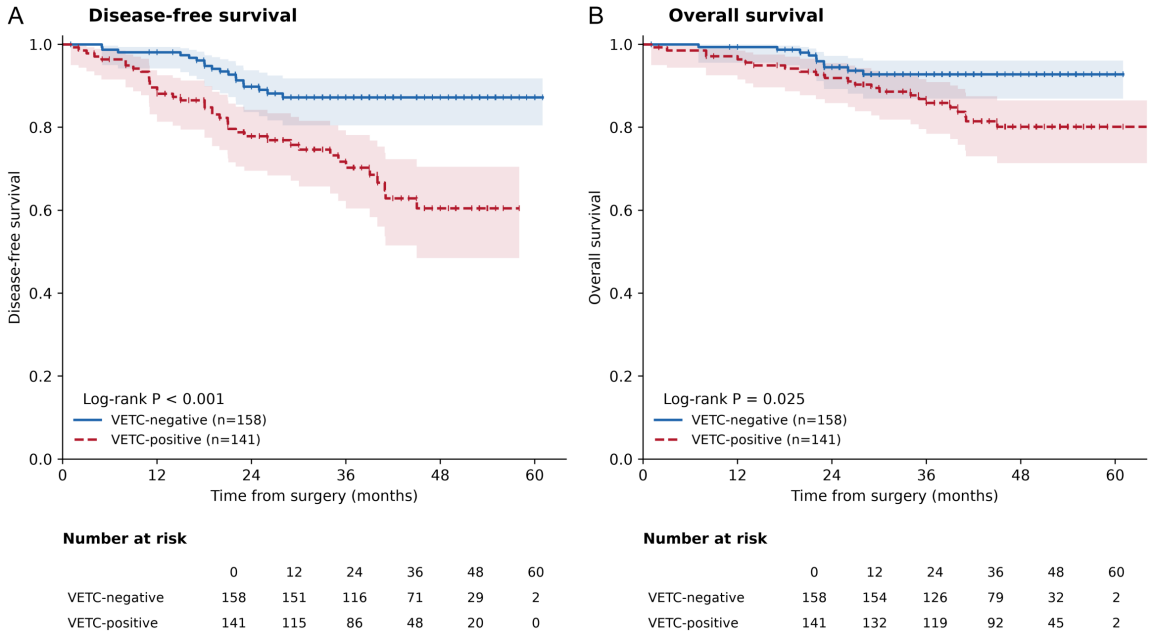


Figure 3. Kaplan-Meier survival curves by VETC status. A. Disease-free survival (DFS), measured from surgery to first recurrence or death. Patients without an event were censored at their last recurrence-free assessment (57 events; log-rank $P < 0.001$). B. Overall survival (OS), measured from surgery to death. Survivors were censored at their last confirmed alive date (33 events; log-rank $P = 0.025$). Shaded regions indicate pointwise 95% confidence intervals (CIs), calculated using Greenwood variance and a log-log transformation. Tick marks indicate censoring, and numbers at risk are shown at 0, 12, 24, 36, 48 and 60 months. Time in months was calculated as days divided by 30.4375. Curves, CIs and risk counts were regenerated from the reconciled dates. Abbreviations: VETC, vessel-encapsulating tumor clusters; LVI, lymphovascular invasion; DFS, disease-free survival; OS, overall survival.

Table 2. Survival analyses

Endpoint	Events	Log-rank *P*	VETC-only HR (95% CI)	Wald *P*
DFS	57	< 0.001	2.88 (1.65-5.03)	< 0.001
OS	33	0.025	2.30 (1.09-4.84)	0.029

Intervals were calculated from surgery to the event or censoring date defined in Section 2.1. Cox models used the Efron method to account for tied event times. Scaled Schoenfeld tests did not detect departures from proportional hazards; numerical results are reported in Section 3.2. The HRs are exploratory estimates of association rather than validated prognostic effects. Post hoc adjustment included VETC and LVI only, with two coefficient degrees of freedom and 299 patients. Events per coefficient were 28.5 for DFS and 16.5 for OS. For DFS, the adjusted VETC HR was 1.43 (95% CI 0.78-2.62; $P = 0.241$). The LVI HR was 5.84 (95% CI 2.33-14.65; $P < 0.001$). For OS, the adjusted VETC HR was 1.57 (95% CI 0.66-3.73; $P = 0.310$). The LVI HR was 2.16 (95% CI 0.77-6.04; $P = 0.141$). These estimates do not establish mediation or independent prognostic validity.

External validation is needed to assess the reproducibility of these associations.

Histological grade, clinical subtype and stage represent distinct aspects of breast cancer characterization [24-27]. Excluding unreconciled grading records limits assessment of confounding by histological grade. Proliferative markers [28], HER2 testing [29] and hormone receptor assessment [30] also require appropriate measurement and interpretation. The lower proportion classified as High in the Postoperative Ki-67 category does not demonstrate a treatment-induced change. FISH alone is insufficient to assign molecular subtype in this cohort.

The retrospective, single-center design and incomplete treatment and molecular-subtype information limit interpretation of the findings. Formal interobserver reliability could not be estimated from the retained pathology records. Quantitative VETC area and histological grade were excluded because of inconsistent recording. Recurrence before a later death may not have been fully ascertained. The DFS endpoint

included observed deaths but did not separately capture second primary cancers. Cox adjustment was limited to LVI, and the modest event counts reduce the sensitivity of proportional-hazards diagnostics. The cell-expression observations remain descriptive because original experimental documentation was unavailable (**Figure 2B**, **2C**). These limitations restrict clinical application and preclude causal interpretation of the findings.

Conclusion

VETC was associated with LVI and shorter unadjusted DFS and OS in this invasive breast cancer cohort. These survival associations were not statistically significant after adjustment for LVI alone. The findings warrant further validation but do not establish independent prognostic utility, treatment prediction or a causal mechanism.

Acknowledgements

This work was supported by the Henan Provincial Science and Technology Key Project; the Henan Province Medical Science and Technology Research Plan jointly created by provincial and ministerial departments (grant numbers SBGJ202502017, SBGJ2022016); the Medical Education Research Project in Henan Province for 2025 (grant number WJLX2025013); the ZZU Education and Teaching Reform Research Project for 2024 (grant number 2024ZZUJGXM263); the Doctoral Research Start-up Fund (grant number ZC20250047) and the Postdoctoral Research Start-up Fund (grant number ZC20250009) from the Henan Provincial People's Hospital; the National Natural Science Foundation of China (grant no. 82503881); the China Postdoctoral Science Foundation (grant number 2025M772151); the Henan Province International Training Program for High-level Talents (grant number GCC2025043); and the Henan Provincial Key Scientific and Technological Project (grant numbers 252102311054 and 262102311081).

Informed consent was obtained from all participants.

Disclosure of conflict of interest

None.

Address correspondence to: Wentao Li and Yuanxiang Lu, Department of Breast Surgery, Henan Provincial People's Hospital, Henan Medical University, No. 7 Weiwu Road, Zhengzhou 450003, Henan, China. E-mail: lwt9225@163.com (WTL); luyx220@163.com (YXL)

References

[1] Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I and Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024; 74: 229-263.

[2] Siegel RL, Miller KD, Wagle NS and Jemal A. Cancer statistics, 2023. CA Cancer J Clin 2023; 73: 17-48.

[3] Siemann DW and Horsman MR. Modulation of the tumor vasculature and oxygenation to improve therapy. Pharmacol Ther 2015; 153: 107-124.

[4] Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med 1971; 285: 1182-1186.

[5] Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.

[6] Weidner N. Intratumor microvessel density as a prognostic factor in cancer. Am J Pathol 1995; 147: 9-19.

[7] Vermeulen PB, Gasparini G, Fox SB, Toi M, Martin L, McCulloch P, Pezzella F, Viale G, Weidner N, Harris AL and Dirix LY. Quantification of angiogenesis in solid human tumours: an international consensus on the methodology and criteria of evaluation. Eur J Cancer 1996; 32A: 2474-2484.

[8] Bergers G and Benjamin LE. Tumorigenesis and the angiogenic switch. Nat Rev Cancer 2003; 3: 401-410.

[9] Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science 2005; 307: 58-62.

[10] Carmeliet P and Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature 2011; 473: 298-307.

[11] Kerbel RS. Tumor angiogenesis. N Engl J Med 2008; 358: 2039-2049.

[12] Fang JH, Zhou HC, Zhang C, Shang LR, Zhang L, Xu J, Zheng L, Yuan Y, Guo RP, Jia WH, Yun JP, Chen MS, Zhang Y and Zhuang SM. A novel vascular pattern promotes metastasis of hepatocellular carcinoma in an epithelial-mesenchymal transition-independent manner. Hepatology 2015; 62: 452-465.

[13] Renne SL, Woo HY, Allegra S, Rudini N, Yano H, Donadon M, Viganò L, Akiba J, Lee HS, Rhee

H, Park YN, Roncalli M and Di Tommaso L. Vessels encapsulating tumor clusters (VETC) is a powerful predictor of aggressive hepatocellular carcinoma. Hepatology 2020; 71: 183-195.

[14] Nielsen TO, Leung SCY, Rimm DL, Dodson A, Acs B, Badve S, Denkert C, Ellis MJ, Fineberg S, Flowers M, Kreipe HH, Laenkholm AV, Pan H, Penault-Llorca FM, Polley MY, Salgado R, Smith IE, Sugie T, Bartlett JMS, McShane LM, Dowsett M and Hayes DF. Assessment of Ki67 in breast cancer: updated recommendations from the International Ki67 in Breast Cancer Working Group. J Natl Cancer Inst 2021; 113: 808-819.

[15] Kaplan EL and Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53: 457-481.

[16] Cox DR. Regression models and life-tables. J R Stat Soc Series B Stat Methodol 1972; 34: 187-202.

[17] Peduzzi P, Concato J, Kemper E, Holford TR and Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. J Clin Epidemiol 1996; 49: 1373-1379.

[18] Vittinghoff E and McCulloch CE. Relaxing the rule of ten events per variable in logistic and Cox regression. Am J Epidemiol 2007; 165: 710-718.

[19] Harrell FE Jr, Lee KL and Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med 1996; 15: 361-387.

[20] Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 1986; 315: 1650-1659.

[21] Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lønning PE and Børresen-Dale AL. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001; 98: 10869-10874.

[22] Mohammed RA, Martin SG, Gill MS, Green AR, Paish EC and Ellis IO. Improved methods of detection of lymphovascular invasion demonstrate that it is the predominant method of vascular invasion in breast cancer and has important clinical consequences. Am J Surg Pathol 2007; 31: 1825-1833.

[23] Mohammed RA, Ellis IO, Elsheikh S, Paish EC and Martin SG. Lymphatic and angiogenic characteristics in breast cancer: morphometric analysis and prognostic implications. Breast Cancer Res Treat 2009; 113: 261-273.

[24] Elston CW and Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 1991; 19: 403-410.

[25] Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B and Senn HJ; Panel members. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol 2013; 24: 2206-2223.

[26] Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO and Botstein D. Molecular portraits of human breast tumours. Nature 2000; 406: 747-752.

[27] Edge SB and Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 2010; 17: 1471-1474.

[28] Colozza M, Azambuja E, Cardoso F, Sotiriou C, Larsimont D and Piccart MJ. Proliferative markers as prognostic and predictive tools in early breast cancer: where are we now? Ann Oncol 2005; 16: 1723-1739.

[29] Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, Allred DC, Bartlett JM, Bilous M, Fitzgibbons P, Hanna W, Jenkins RB, Mangu PB, Paik S, Perez EA, Press MF, Spears PA, Vance GH, Viale G and Hayes DF; American Society of Clinical Oncology; College of American Pathologists. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol 2013; 31: 3997-4013.

[30] Hammond ME, Hayes DF, Wolff AC, Mangu PB and Temin S. American society of clinical oncology/college of American pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol 2010; 6: 195-7.

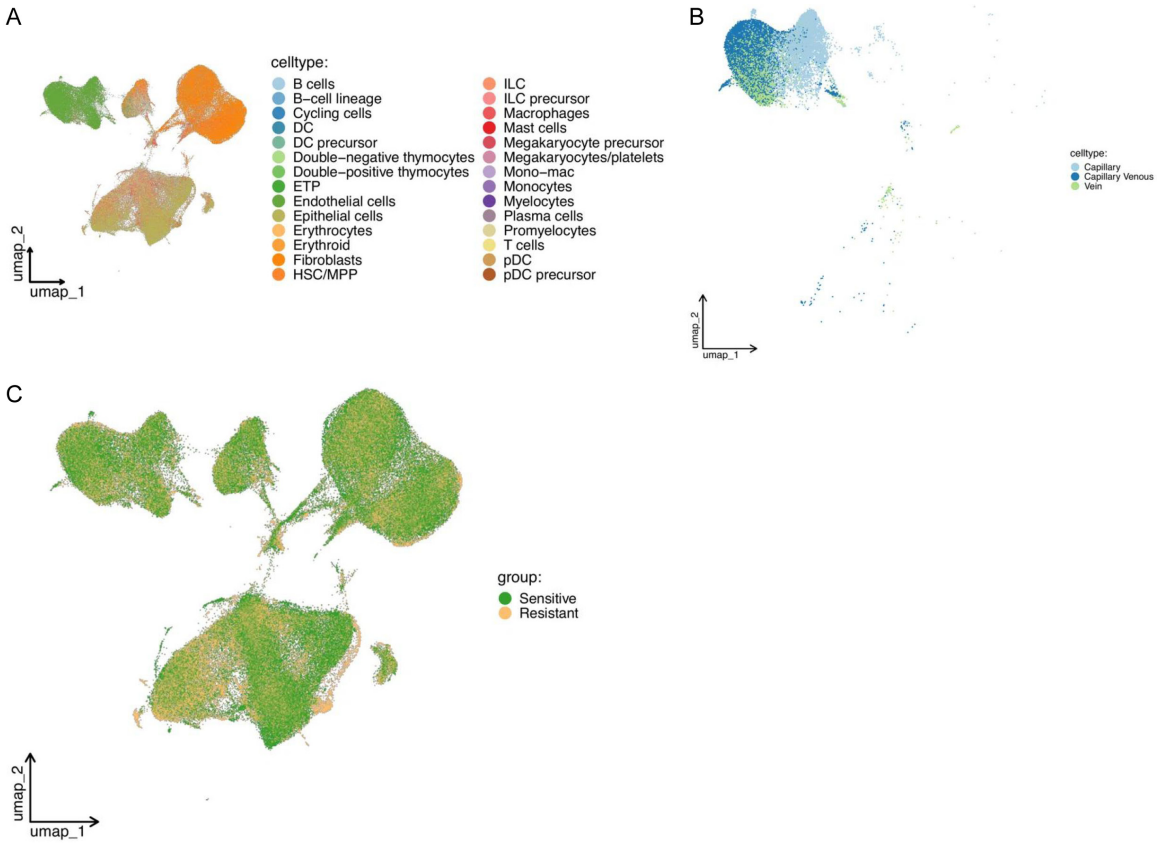


Figure S1. Exploratory single-cell transcriptomic analysis of endothelial-cell heterogeneity in breast cancer. (A) Uniform manifold approximation and projection (UMAP) of single-cell RNA-sequencing data from breast cancer tissue, coloured by annotated cell population. Twenty-nine populations were annotated, including B cells and B-cell lineage, T cells, innate lymphoid cells (ILC) and ILC precursors, thymocyte subsets (double-negative, double-positive and early thymic progenitor, ETP), myeloid populations (dendritic cells and dendritic-cell precursors, macrophages, monocytes, mono-mac, neutrophils, myelocytes, promyelocytes, mast cells, megakaryocyte precursors, megakaryocytes/platelets, plasmacytoid dendritic cells and their precursors), plasma cells, cycling cells, endothelial cells, epithelial cells, fibroblasts, erythroid cells and haematopoietic stem cells/multipotent progenitors (HSC/MPP). Each colour denotes one annotated population. (B) UMAP of the endothelial compartment, resolving three endothelial subsets: capillary, capillary-venous and venous endothelial cells. (C) The same embedding as in (A), coloured by treatment-response group (sensitive, green; resistant, orange). Projections are shown for descriptive purposes.