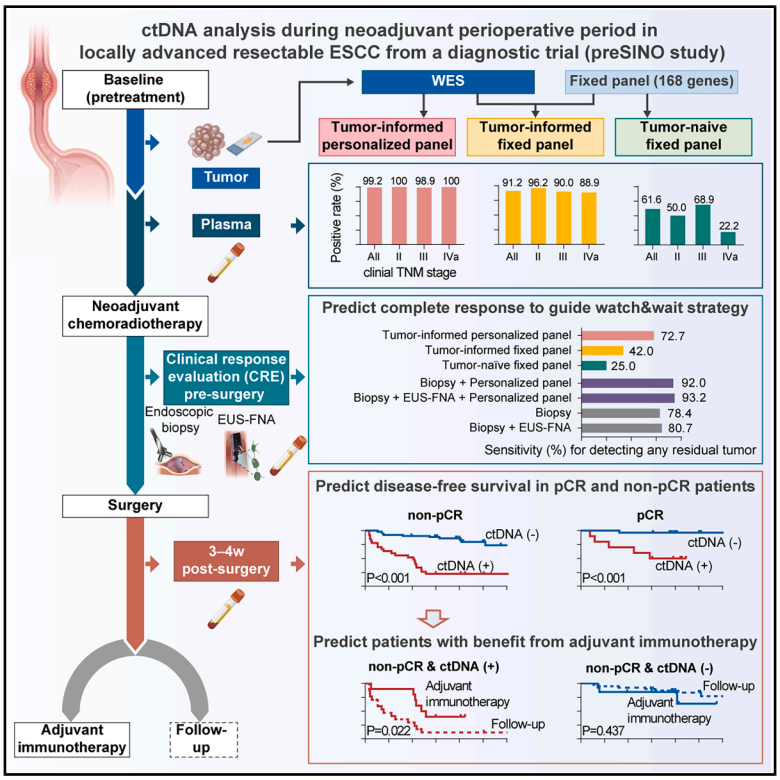
Cell Reports
Medicine

Article

ctDNA detects residual disease after neoadjuvant chemoradiotherapy and guides adjuvant therapy in esophageal squamous cell carcinoma

Graphical abstract



Highlights

- Personalized ctDNA assay shows higher MRD sensitivity than fixed panels in ESCC
- Adding ctDNA to clinical evaluation improves detection of non-pCR patients post-nCRT
- Post-surgical ctDNA predicts recurrence and identifies benefit from adjuvant therapy
- ctDNA-MRD detection supports organ-sparing strategies and adaptive adjuvant therapy

Authors

Zhichao Liu (刘智超), Guoqiang Wang (王国强), Yang Yang (杨洋), ..., Chunguang Li (李春光), Shangli Cai (蔡尚立), Zhigang Li (李志刚)

Correspondence

dr_chunguang_li@shsmu.edu.cn (C.L.), shangli.cai@brbiotech.com (S.C.), zhigang.li@shsmu.edu.cn (Z.L.)

In brief

Accurate detection of residual disease after neoadjuvant therapy in ESCC remains an unmet need. Liu et al. reveal that integrating ctDNA with clinical response evaluation improves residual disease detection post-neoadjuvant chemoradiotherapy to support organ-sparing strategies and that postoperative ctDNA stratifies recurrence risk beyond pathological response to inform adjuvant immunotherapy decisions.

Liu et al., 2025, Cell Reports Medicine 6, 102334
September 16, 2025
https://doi.org/10.1016/j.xcrm.2025.102334

CellPress

Cell Reports Medicine

CellPress
OPEN ACCESS

Article

ctDNA detects residual disease after neoadjuvant chemoradiotherapy and guides adjuvant therapy in esophageal squamous cell carcinoma

Zhichao Liu (刘智超),[1,2,10] Guoqiang Wang (王国强),[3,10] Yang Yang (杨洋),[1,10] Yuchen Su (苏瑜琛),[4] Hong Zhang (章宏),[4] Jun Liu (刘俊),[5] Peng Cui (崔鹏),[3] Xuning Fan (范旭宁),[3] Jinyu Yang (杨瑾裕),[3] Zhihong Zhang (张之宏),[3] Xing Gao (高杏),[6,7] Yinkai Chao (赵盈凯),[7] Bianca Mostert,[8] J. Jan B. van Lanschot,[6] Bas P.L. Wijnhoven,[6] Simon Law (罗英杰),[9] Chunguang Li (李春光),[1,2,*] Shangli Cai (蔡尚立),[3,*] and Zhigang Li (李志刚)[1,2,11,*]

[1]Department of Thoracic Surgery, Shanghai Key Laboratory of Thoracic Tumor Biotherapy, Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200030, China
[2]Shanghai Institute of Thoracic Oncology, Shanghai 200030, China
[3]Burning Rock Biotech, Guangzhou 510300, China
[4]Center for Esophageal Diseases, Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200030, China
[5]Department of Radiation Oncology, Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200030, China
[6]Department of Surgery, Erasmus MC Cancer Institute, Erasmus University Medical Centre, 3015GD Rotterdam, the Netherlands
[7]Division of Thoracic Surgery, Chang Gung Memorial Hospital-Linkou, Chang Gung University, Taiwan 33305, China
[8]Department of Medical Oncology, Erasmus MC Cancer Institute, Erasmus University Medical Centre, 3015GD Rotterdam, the Netherlands
[9]Department of Surgery, LKS Faculty of Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong 999077, China
[10]These authors contributed equally
[11]Lead contact
*Correspondence: dr_chunguang_li@shsmu.edu.cn (C.L.), shangli.cai@brbiotech.com (S.C.), zhigang.li@shsmu.edu.cn (Z.L.)
https://doi.org/10.1016/j.xcrm.2025.102334

SUMMARY

The diagnostic accuracy of circulating tumor DNA (ctDNA) for detecting molecular residual disease (MRD) after multimodal treatment remains unclear. In a prospective cohort of 132 patients with locally advanced esophageal squamous cell carcinoma (ESCC) undergoing neoadjuvant chemoradiotherapy (nCRT) followed by clinical response evaluation and surgery, tumor-informed personalized-panel and fixed-panel ctDNA assays are applied to serial blood samples. Personalized ctDNA assay demonstrates a superior baseline detection rate (99.2%) and outperforms fixed panels in diagnosing post-nCRT residual disease. Integrating personalized ctDNA with conventional clinical diagnostic methods increases sensitivity for predicting non-pathological complete response (non-pCR) from 78.4%–80.7% to 92.0%–93.2%. Patients with detectable MRD post-nCRT and/or post-surgery exhibit worse survival outcomes. In non-pCR patients, adjuvant immunotherapy improves disease-free survival in post-surgery MRD-positive cases, whereas MRD-negative patients derive no benefit. These findings support incorporating ctDNA into response assessment to guide organ-sparing strategies and adjuvant therapy decisions in ESCC. This study is registered at ClinicalTrials.gov (NCT03937362).

INTRODUCTION

Esophageal cancer is the seventh leading cause of cancer-related mortality worldwide, with esophageal squamous cell carcinoma (ESCC) accounting for nearly 90% of cases.[1,2] Neoadjuvant chemoradiotherapy (nCRT) followed by surgery is a standard treatment for locally advanced esophageal cancer.[3,4] However, a substantial proportion of patients achieve a pathological complete response (pCR) after nCRT, particularly in ESCC (~40%), raising questions about the necessity of standard esophagectomy for all patients.[5,6] Intuitively, surgery may not offer oncological benefit for patients with no viable residual tumor. Therefore, for patients who show no signs of residual disease after nCRT (defined as clinically complete response [cCR]), a "Watch and Wait" strategy—also referred to organ-sparing approach with active surveillance—has been proposed as an alternative to standard esophagectomy, given the considerable morbidity and mortality risks associated with esophagectomy, which can severely compromise quality of life.[7,8]

A critical challenge in implementing organ preservation strategies is the accurate detection of residual disease after neoadjuvant therapy.[5] The European preSANO (pre-surgery as needed for oesophageal cancer) trial established a clinical response evaluation (CRE) strategy using clinical diagnostic methods of endoscopic bite-on-bite biopsy of primary lesion and endoscopic ultrasound with fine needle aspiration (EUS-FNA) of suspected lymph nodes.[2] However, this conventional CRE strategy, despite a 10% false-negative rate for detecting major residual disease (referred to non-major pathological response [non-MPR], defined as >10% viable residual cancer), exhibited a

concerning 23% false-negative rate for detecting any residual disease (referred to non-pCR, defined as ≥1% viable residual cancer), suggesting its insufficiency to detect minimal residual tumors. This limitation was further confirmed in the phase 3 SANO (surgery as needed for oesophageal cancer) trial. While showing non-inferior overall survival for active surveillance versus standard surgery in cCR patients evaluated by conventional CRE strategy post-nCRT, the trial revealed that 43% of patients who underwent active surveillance developed distant metastases compared to 34% of those who underwent standard surgery.[9,10] These findings highlight the limitations of conventional diagnostic tools in detecting molecular residual disease (MRD) and emphasize the need for more sensitive biomarkers.

Circulating tumor DNA (ctDNA) has emerged as a promising tool for detecting MRD and predicting recurrence in several tumor types.[11,12] ctDNA assays are generally categorized into tumor-naive (agnostic) assay, which adopts no prior information about tumor mutations in the assay, and tumor-informed assay, which incorporates the genetic information from the corresponding tumor tissue. Tumor-informed assay can be further divided into personalized and fixed-panel assays. The MEDAL (methylation based dynamic analysis for lung cancer) study and the TRACEx (tracking non-small cell lung cancer evolution through therapy [Rx]) study have shown that personalized tumor-informed ctDNA assay has excellent sensitivity in MRD detection in lung cancer.[13,14] For colorectal cancer, both the DYNAMIC study and the GALAXY (Genetic alterations and clinical record in radically resected colorectal cancer revealed by liquid biopsy and whole exome analysis) study demonstrated the utility of ctDNA for postoperative risk stratification and for guiding adjuvant treatment.[11,15] Additionally, undetectable ctDNA after neoadjuvant therapy has been associated with improved outcomes in lung cancer and in breast cancer.[16–18] These studies highlight the potential of ctDNA in neoadjuvant response assessment for adaptive treatment. However, ctDNA remains underexplored in residual disease detection after neoadjuvant therapy for esophageal cancer. Two pivotal questions remain: first, for patient selection after nCRT for organ-sparing strategies, it is still unclear whether ctDNA can enhance the detection of residual disease, either alone or in combination with conventional clinical diagnostic modalities; second, for patients undergoing standard surgery post-nCRT, the role of ctDNA remains unclear in postoperative management, particularly in guiding adjuvant immunotherapy.

In this study, we explored the value of ctDNA as a molecular response evaluation (MoRE) strategy to improve residual disease detection during neoadjuvant and perioperative decision-making for ESCC patients from a prospective diagnostic trial (preSINO, pre-surgery if needed for oesophageal cancer). We assessed the clinical validity of ctDNA for identifying complete responders after nCRT to support organ-sparing strategies and for stratifying postoperative recurrence risk to guide adjuvant immunotherapy decisions.

RESULTS

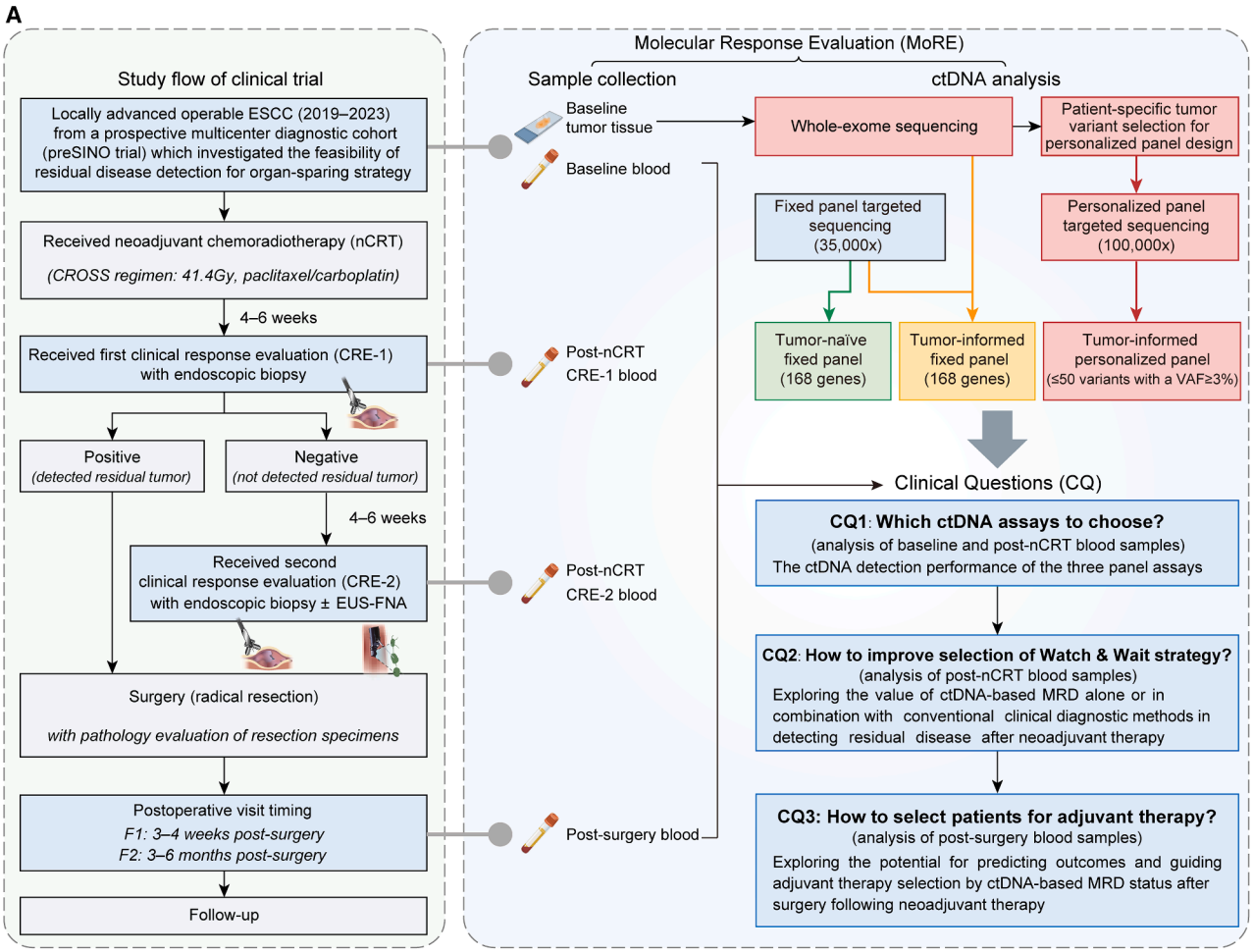
Patient characteristics

The study design is illustrated in Figure 1A (STAR Methods). Between August 2019 and January 2023, 242 patients with locally advanced ESCC who underwent nCRT followed by standard CRE and surgery were included in the preSINO diagnostic cohort study.[19] Of these, 132 patients (biomarker evaluable set [BES]) were prospectively recruited for ctDNA analysis. Reasons for exclusion from ctDNA analysis are detailed in Figure S1. Patient characteristics were comparable between BES and full analysis set including age, gender, tumor location, clinical TNM (tumor, node, metastasis) stage, and neoadjuvant therapy response (all $p > 0.05$, Table S1). Additionally, no significant differences in clinical characteristics or pathological response were observed between ctDNA-evaluable patients (BES) and ctDNA-non-evaluable patients (all $p > 0.05$, Table S1). Among the 132 ctDNA-evaluable patients, a total of 43 patients (32.6%) achieved pCR (defined as the absence of residual tumor) after nCRT. Fifteen patients had 1%–10% residual tumor, and, when combined with the 43 cases with pCR, 58 patients (43.9%) were classified as having a major pathological response (MPR, defined as 0%–10% residual tumor). All patients were confirmed without distant metastases by preoperative positron emission tomography (PET)-computed tomography (CT) and underwent complete resection. After nCRT, all patients underwent one or two CREs as specified by standard clinical diagnostic approach using endoscopic biopsy and EUS-FNA. Detailed CRE results and pathological diagnoses are shown in Figure S2. At CRE-1 time point, 56 patients (42.4%) were evaluated as positive by endoscopic biopsy and proceeded directly to surgery, among whom 53 patients (94.6%) were non-pCR. Of the 76 patients who proceeded to CRE-2 with endoscopic biopsy and EUS-FNA 4–6 weeks after CRE-1, 18 (23.7%) were evaluated as positive with residual disease and 58 (76.3%) were negative. Among the 18 CRE-2 positive patients, all were non-pCR, while, among the 58 CRE-2 negative patients, 40 (69.0%) were pCR. A total of 489 blood samples from the 132 patients were included in the ctDNA analysis (Figure 1B).

Comparison of performance of the three ctDNA assays to detect MRD

Benchmarking ctDNA detection rates at baseline (pretreatment) is essential for evaluating assay sensitivity, particularly when interpreting treatment response after neoadjuvant therapy and informing subsequent decisions regarding adjuvant treatment. In this study, we applied three previously validated ctDNA assays[13] for MRD detection: tumor-informed personalized assay, tumor-informed fixed assay, and tumor-naive fixed assay. For the tumor-informed personalized assay, up to 50 highly ranked variants were identified from the whole-exome sequencing data of the tumor tissues to form a personalized panel.[13] The tumor-naive fixed assay used a fixed panel covering 168 cancer-related genes previously validated in the Food and Drug Administration-led sequencing quality control phase 2 (SEQC2) project.[20] For the tumor-informed fixed assay, the same fixed panel was used, but any SNV/insertion or deletion not detected in the paired tumor tissue was excluded, resulting in a lower reported allele frequency than the tumor-naive fixed assay. Detailed information for the three assays is provided in STAR Methods and Tables S2 and S3. We first compared assay sensitivity by evaluating baseline ctDNA detection rates among patients with paired ctDNA results from all three assays ($n = 125$).

CellPress
OPEN ACCESS



Time points		Patients received clinical response evaluation by clinical diagnostic approach using endoscopy biopsy and EUS-FNA	Patients with available ctDNA analysis			
			Tumor-informed personalized assay	Tumor-informed fixed assay	Tumor-naïve fixed assay	With results for the three ctDNA assays
pretreatment	Baseline	/	132	125	128	125
post-nCRT	CRE-1 *(4–6 weeks post-nCRT)*	132	131	129	130	129
	CRE-2 *(10–12 weeks post-nCRT)*	76	70	70	71	69
post-surgery	F1 *(3–4 weeks post-surgery)*	/	116	/	/	/
	F2 *(3–6 months post-surgery)*	/	40	/	/	/

Figure 1. Study schematic

(A) Flowchart of the study design.

(B) Number of patients with available ctDNA data at each predefined time point. See also Figures S1 and S2 and Table S1.

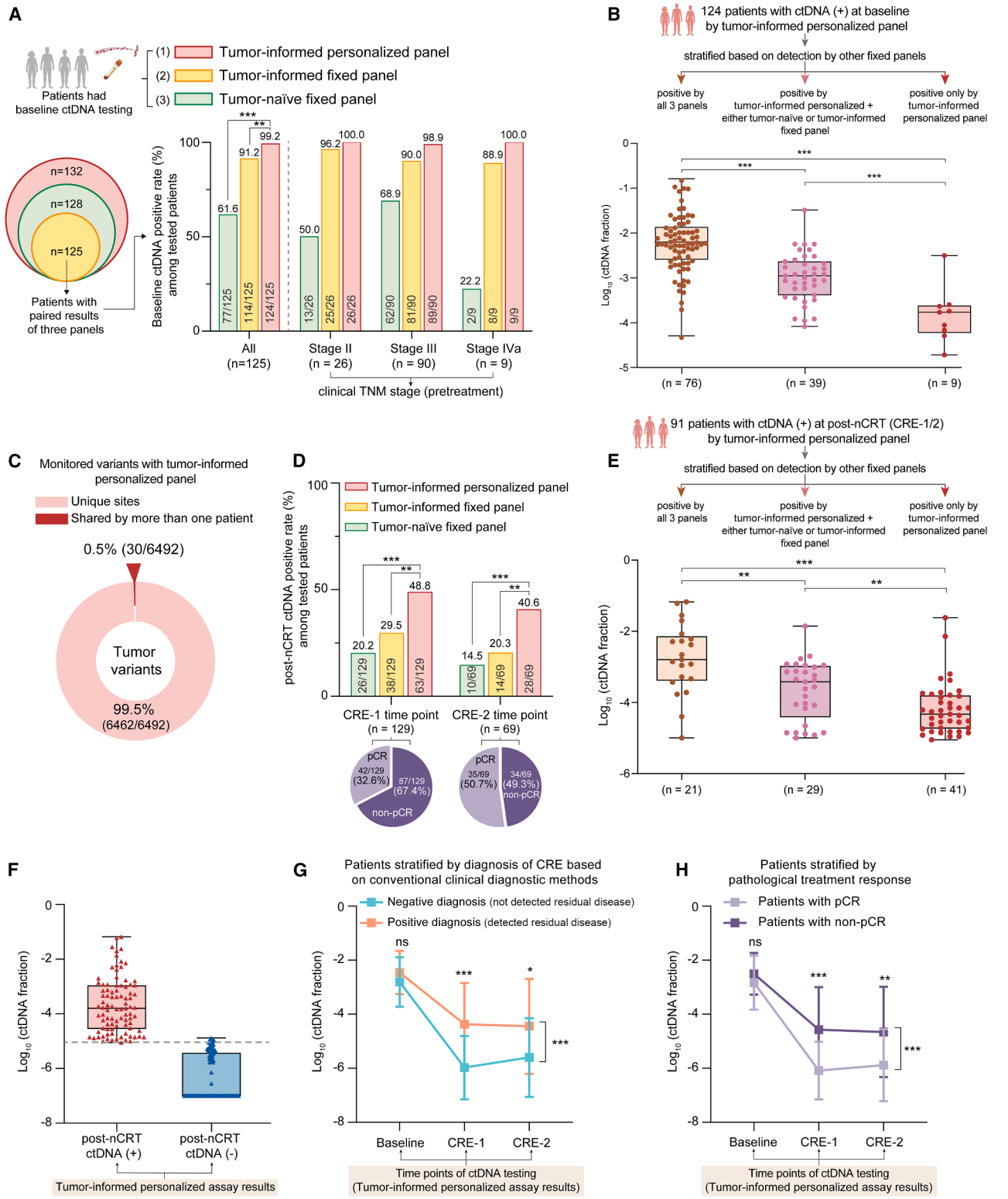


Figure 2. Comparison of tumor-informed personalized and fixed-panel ctDNA assays for MRD detection before and after nCRT

(A) ctDNA-positive detection rates of three assays at baseline, calculated as the proportion of ctDNA-positive patients among all tested individuals. See also Tables S2 and S3.

(legend continued on next page)

The positive detection rate at baseline was significantly higher with the tumor-informed personalized assay than the other assays (99.2% vs. 91.2% vs. 61.6%, $p < 0.001$, Figure 2A). When stratified by tumor stage, the tumor-informed personalized assay showed higher ctDNA-positive rates across all subgroups compared to the other assays. Among patients with detectable ctDNA by tumor-informed personalized assay at baseline, those who were positive only by this assay had significantly lower ctDNA fractions than those who were also positive by either one or both fixed-panel assays (Figure 2B). This highlights the personalized assay's sensitivity to detect low-level ctDNA that might be missed by fixed-panel assays, which is important in the post-treatment setting where ctDNA levels are low. Notably, most variants (99.5%) tracked by the tumor-informed personalized assay were unique to individual patients (Figure 2C), highlighting the substantial inter-patient heterogeneity in ESCC and the limitations of hotspot-based fixed panels. When compared with previously published custom fixed-panel MRD studies, only a small proportion (2.4%–10.0%, Table S4) of the variant sites in our cohort were covered by those fixed panels,[21–25] which may explain the lower ctDNA-positive rates (37.5%–75.5%) reported in prior studies for esophageal cancer.

We next evaluated ctDNA detection at post-nCRT time points (CRE-1 and CRE-2) following a diagnostic trial design to evaluate the feasibility of organ-sparing strategies (Figure 1A, STAR Methods). After nCRT, patients underwent one or two CRE (Figure S2). Those with a positive diagnosis at the first evaluation (CRE-1, endoscopic biopsy performed 4–6 weeks post-nCRT) proceeded to immediate surgery, while those with a negative result proceeded to second evaluation (CRE-2, endoscopic biopsy and EUS-FNA at 10–12 weeks post-nCRT) before surgery. As shown in Figure 2D, the tumor-informed personalized assay maintained a significantly higher positive rate at both CRE-1 (48.8% vs. 20.2% and 29.5%) and CRE-2 (40.6% vs. 14.5% and 20.3%) time points compared with the other assays. This decline in ctDNA detection rates compared to baseline reflects true biological response to neoadjuvant therapy, rather than assay insensitivity, as a substantial proportion of patients who underwent ctDNA testing and achieved pCR after nCRT (32.6% at CRE-1 and 50.7% at CRE-2, see pie charts in Figure 2D). Among patients with ctDNA positivity detected by tumor-informed personalized assay at post-nCRT (CRE-1/2), those who were positive only by this assay had significantly lower ctDNA fractions than those who were also positive by either one or both fixed-panel assays (Figure 2E), consistent with findings at baseline. This further supports the superior sensitivity of personalized assay in detecting MRD under conditions of reduced tumor burden after neoadjuvant therapy. Notably, ctDNA fraction detected by the tumor-informed personalized assay reached as low as 0.004% (Figure 2F).

We also explored the association between ctDNA fractions and treatment response, using both clinical evaluation and pathological response. At baseline, there was no difference in ctDNA fraction between patients classified as CRE positive and CRE negative by conventional diagnostic methods (Figure 2G). However, post-nCRT ctDNA fractions at both the CRE-1 and CRE-2 time points were significantly higher in patients with clinically diagnosed residual disease compared to those without. A similar pattern was observed when patients were stratified by pathological response (Figure 2H): patients with non-pCR had significantly higher ctDNA fractions than those with pCR at both CRE-1 and CRE-2 time points. These findings support the sensitivity of the tumor-informed personalized assay for MRD detection and its correlation with treatment response.

Integrated ctDNA and clinical assessment improve sensitivity for residual disease detection after nCRT

We next assessed the feasibility of ctDNA-based MoRE in predicting residual disease after nCRT. A total of 131 patients with post-nCRT ctDNA data from all three assays were included for analysis. For each patient, diagnostic information from both CRE-1 and CRE-2 time points was integrated to generate a final prediction (Figure 3A): a positive result at either CRE-1 or CRE-2 by a diagnostic test (e.g., conventional endoscopic biopsy or ctDNA assay) was classified as "predicted positive" for residual disease, while negative results at both time points were classified as "predicted negative." This two-step assessment approach reflects real-world clinical practice and has been demonstrated in prior trials[10,26] as a feasible strategy to identify low-risk patients for organ preservation.[5] We then evaluated the accuracy of each diagnostic modality to identify patients with pathological residual disease (non-pCR), using surgical histopathology as the reference standard. Sensitivity was defined as the proportion of non-pCR patients who tested positive by each diagnostic method. The tumor-informed personalized assay outperformed the other two assays in predicting non-pCR (any residual tumor), with a sensitivity of 72.7% compared to 25.0%

(B) Boxplots comparing ctDNA fractions at baseline among patients with ctDNA detected by the tumor-informed personalized panel, stratified by additional detection by fixed panels: all three assays (brown), personalized plus either fixed panel (pink), or personalized only (red).
(C) Pie chart showing the proportion of unique and shared tumor variants tracked by the tumor-informed personalized panel among 132 ESCC patients. Most variants were unique to individual patients, while only a small proportion were shared by more than one patient. See also Table S4.
(D) ctDNA-positive detection rates of the three assays at post-nCRT time points (CRE-1/2), calculated as the proportion of ctDNA-positive patients among all tested individuals. Pie charts below show the proportion of patients with pCR and non-pCR among those who underwent ctDNA testing at each time point.
(E) Boxplots comparing ctDNA fractions at post-nCRT (CRE-1/2) among patients with ctDNA detected by the tumor-informed personalized panel, stratified by additional detection by fixed panels: all three assays (brown), personalized plus either fixed panel (pink), or personalized only (red).
(F) Boxplot comparing ctDNA fraction between ctDNA-positive and ctDNA-negative patients at post-nCRT (CRE-1/2), as determined by the tumor-informed personalized panel.
(G) Dynamic changes in ctDNA fraction stratified by clinical evaluation (clinically diagnosed residual disease vs. those without) from baseline to post-nCRT (CRE-1 and CRE-2 time points).
(H) Dynamic changes in ctDNA fraction stratified by pathological response (pCR vs. non-pCR) from baseline to post-nCRT (CRE-1 and CRE-2 time points).
ns, not significant; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, by chi-squared test (A and D) or Mann-Whitney test (B and E) or two-way ANOVA in (G and H). Boxplots in (B), (E), and (F) represent median ± interquartile range, and whiskers indicate full range of values.

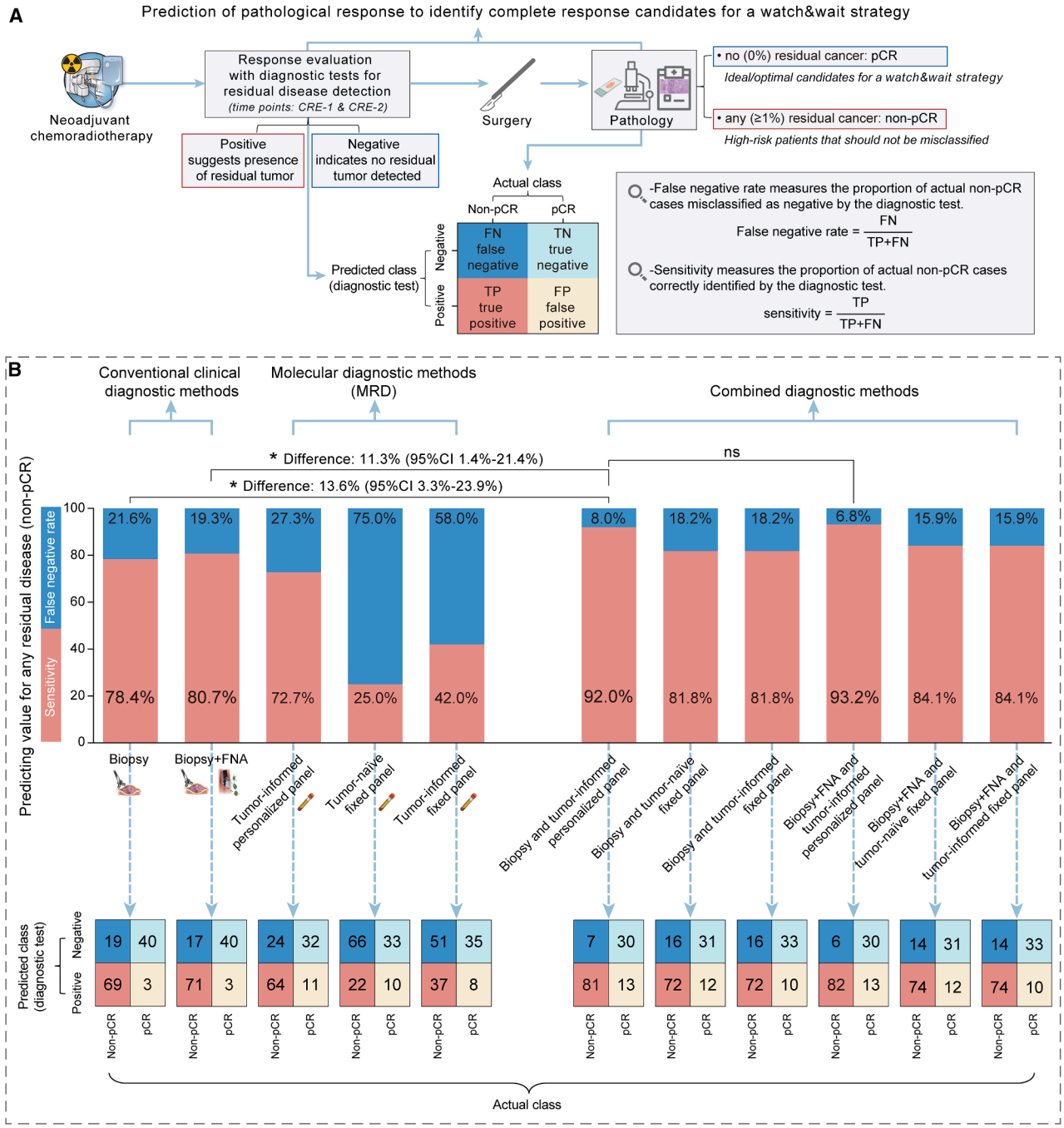


Figure 3. Integrated ctDNA and clinical assessment improve prediction of residual disease and complete response after nCRT

(A) Schematic of the diagnostic workflow for response evaluation. Diagnostic test performance was summarized using a confusion matrix to calculate sensitivity and false-negative rate.

(B) Sensitivity and false-negative rates for detecting any residual disease (non-pCR) using conventional clinical methods, ctDNA-based molecular methods, and their combinations. Absolute numbers used for each calculation are displayed beneath each bar.

*$p < 0.05$ of Fisher's exact test; ns, not significant. See also Table S5 and Figure S3.

and 42.0% for the tumor-naive and tumor-informed fixed panels, respectively (Figure 3B). A similar trend was observed when using an alternative pathological response definition commonly used in neoadjuvant trials—non-MPR, defined as >10% residual tumor and/or presence of nodal metastasis. Compared to the pCR/non-pCR definition, which represents the most stringent threshold for complete response, the MPR-based classification allows for minor residual disease. Under this criterion, the

tumor-informed personalized assay again showed higher sensitivity than the other two fixed-panel assays (79.5% vs. 30.1% and 49.3%, Table S5). Notably, combining the tumor-informed personalized ctDNA assay with conventional clinical diagnostic methods further improved sensitivity for predicting non-pCR from 78.4%–80.7% to 92.0%–93.2%, outperforming the combination with the other ctDNA assays (Figure 3B; Table S5). These findings suggest the potential clinical value of integrating MoRE (referred to ctDNA-based MRD assessment) with conventional CRE (referred to clinical diagnostics of endoscopic biopsy and/or EUS-FNA) to enhance residual disease detection after nCRT, thereby reducing the risk of missed diagnoses of residual disease and supporting safer organ-sparing selection.

Given its superior performance in MRD detection, we focused subsequent analyses on the tumor-informed personalized ctDNA assay. Although the addition of ctDNA to CRE improved sensitivity, an increase in false-positive rate was observed (Figure 3B). We next analyzed additional positive cases contributed by ctDNA testing, stratified by non-pCR (true positives) and pCR (false positives). Among 89 non-pCR patients (Figure S3A), 53 were CRE-1 positive (by endoscopic biopsy), of whom 41 were also ctDNA positive, indicating strong concordance between endoscopic biopsy-based CRE and ctDNA-based MoRE. Of the 36 non-pCR patients with negative CRE-1 diagnosis, 13 (36.1%) were ctDNA positive at the same time point; these patients were not identified by conventional clinical evaluation but could have been considered with residual disease based on ctDNA positivity, potentially enabling timely surgery that would otherwise be delayed by 4–6 weeks until the second CRE. Notably, only 7 of these 13 patients were subsequently identified as positive by clinical assessment at CRE-2. Given that the NeoRes II trial[27] showed that surgery delays (>10 weeks post-nCRT) are associated with worse survival in patients with poor response to nCRT, our findings highlight the potential of integrating ctDNA detection to help prevent unnecessary surgery delays in non-clinical responders missed by CRE-1. Of the remaining 23 patients who were negative by both biopsy and ctDNA at CRE-1 time point, 11 were found to be CRE-2 positive by endoscopic biopsy with EUS-FNA; of the 12 patients who remained CRE-2 negative, 5 were identified as ctDNA positive. Overall, adding MoRE with ctDNA analysis to conventional CRE enabled the identification of 11 additional non-pCR patients (11/89, 12.4%).

Among 43 pCR patients, 3 had CRE-1-positive diagnosis (2 due to endoscopically non-traversable stenosis and 1 due to high-grade dysplasia), and 1 of these 3 patients was ctDNA positive (Figure S3B). Among the remaining 40 patients with CRE-1-negative diagnosis, 8 were ctDNA positive at CRE-1 time point and 2 were ctDNA positive at CRE-2 time point. In total, 13 pCR patients had either positive CRE or positive ctDNA findings post-nCRT and were considered potential false positives. Specifically, 10 patients had ctDNA positivity only, 2 had CRE-1 positivity only, and 1 had both ctDNA and CRE-1 positivity (Figure S3C). Recurrence analysis showed that pCR patients with positive ctDNA post-nCRT had significantly higher recurrence rates (36.4% vs. 6.3%, $p = 0.01$, Figure S3D) than those with negative ctDNA. Moreover, patients with positive ctDNA post-nCRT had worse disease-free survival (DFS) and overall survival (OS) (Figures S3E and S3F). These results suggest that ctDNA can supplement conventional clinical diagnostic methods not only to detect locoregional residual disease (LRD) for predicting non-pCR patients but also to detect systemic residual disease for identifying patients with an increased risk of distant recurrence even among those who achieve a pCR.

Postoperative ctDNA predicts prognosis after nCRT

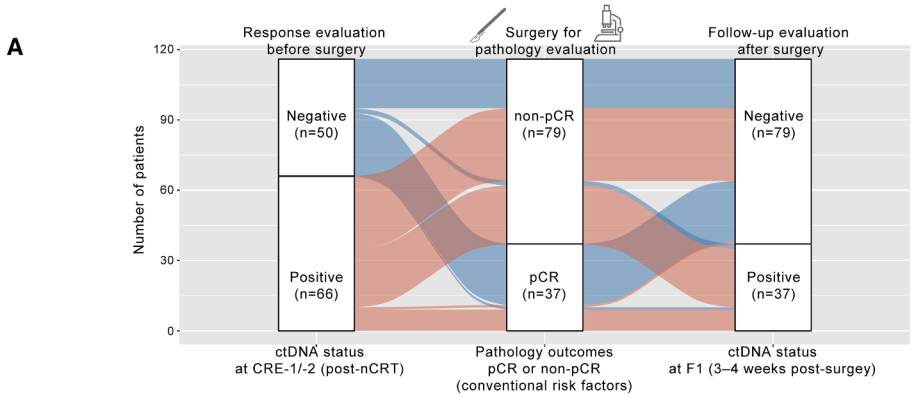
We next evaluated the prognostic value of postoperative ctDNA-based MRD in ESCC following nCRT. We first assessed the dynamic change in ctDNA status from post-nCRT (CRE-1/2) to post-surgery (F1) (Figure 4A). Among the 66 patients with positive ctDNA at post-nCRT time point, 56 were non-pCR and 10 were pCR. Notably, 9 out of the 10 pCR patients (90.0%) remained ctDNA positive at F1, suggesting a possible presence of residual disease beyond the locoregional tumor site. Among the 56 non-pCR patients, 31 (55.4%) converted to ctDNA negativity at F1, indicating that their initial positive ctDNA post-nCRT was likely attributable to LRD that was subsequently removed by surgery. The remaining 25 non-pCR patients (44.6%) remained ctDNA positive at F1, suggesting the potential presence of residual disease beyond locoregional tumor site despite radical surgery. In contrast, among 50 patients with negative ctDNA at post-nCRT time point, most (47/50, 94.0%) remained negative at F1, with 3 patients (6.0%) converted to ctDNA positive at F1 (2 with non-pCR and 1 with pCR). Changes in ctDNA status from post-nCRT to F1 were categorized into four groups: consistent negative ($n = 47$), converted negative ($n = 32$), converted positive ($n = 3$), and consistent positive ($n = 34$). As expected, patients with consistent positive ctDNA had worse DFS and OS (Figures 4B and 4C) compared to those with consistent negative or converted negative ctDNA (Log rank p for trend < 0.001).

Next, we explored whether post-surgical ctDNA could predict recurrence and prognosis. Patients with positive ctDNA at F1 had significantly worse DFS ($p < 0.001$; hazard ratio [HR], 8.80; 95% confidence interval [CI], 4.28–18.11, Figure 4D) and OS ($p < 0.001$; HR, 10.03; 95% CI, 3.92–25.65, Figure 4E) compared to ctDNA-negative patients. Multivariable Cox regression analysis confirmed that post-surgical ctDNA positivity at F1 was an independent prognostic factor for worse survival outcomes after adjustment for clinical variables (age, gender, baseline tumor stage, pCR status, and adjuvant therapy) (Figure 4F). Consistently, positive ctDNA at F2 was associated with worse DFS ($p < 0.001$; HR, 17.07; 95% CI, 4.72–61.68, Figure S4A) and OS ($p < 0.001$; HR, 13.39; 95% CI, 2.91–61.58, Figure S4B). Longitudinal postoperative ctDNA status (F1 and F2) also predicted higher disease progression ($p < 0.001$; HR, 15.58; 95% CI, 6.43–37.78, Figure S4C) and mortality ($p < 0.001$; HR, 12.76; 95% CI, 4.33–37.59, Figure S4D). Furthermore, patients with consistently positive or converted positive ctDNA from F1 to F2 had worse DFS and OS compared to those with consistent or converted negative ctDNA (Log rank p for trend < 0.001, Figures S4E and S4F). These findings support the prognostic value of postoperative ctDNA in ESCC patients treated with nCRT and surgery.

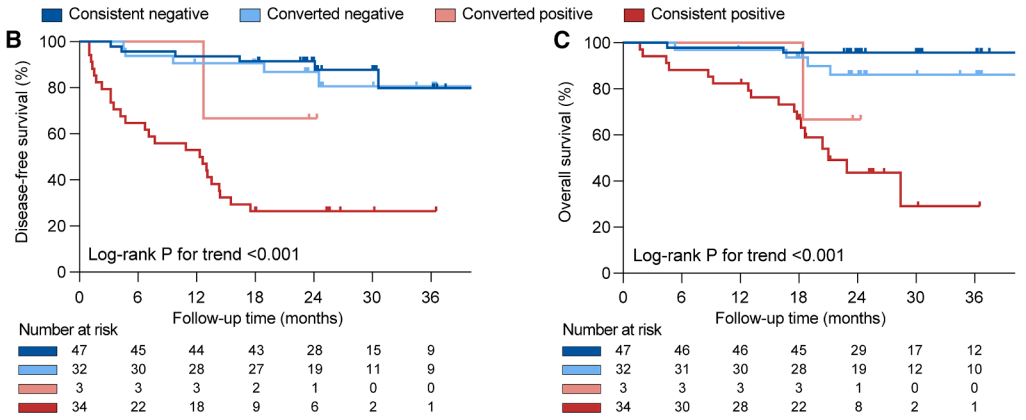
Postoperative ctDNA predicts benefit from adjuvant immunotherapy

Pathological response to neoadjuvant therapy is associated with recurrence and prognosis and currently serves as the primary

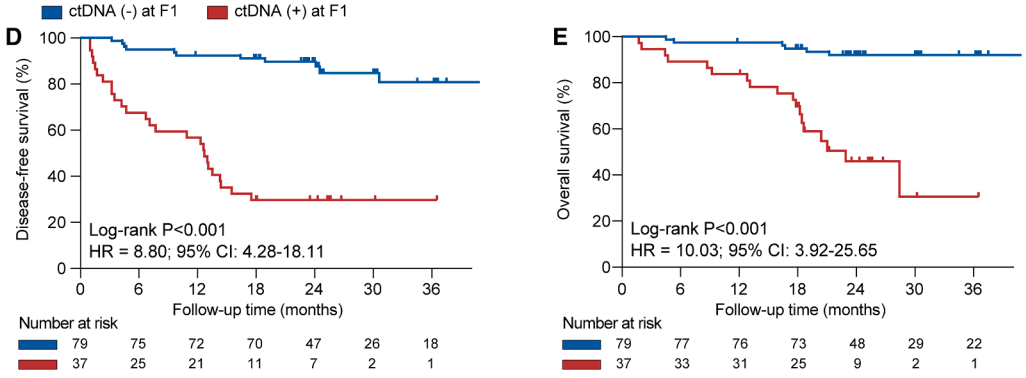
ctDNA-based MRD status (by the tumor-informed personalized panel) from post-nCRT to post-surgery time points.



Dynamic ctDNA change from post-nCRT(CRE-1/-2) to post-surgery (F1)

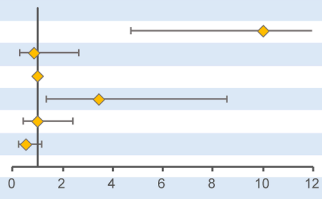


Post-surgery ctDNA at F1 time point (3–4 weeks after surgery)



F

Multivariable Cox regression analysis		Adjusted Hazard Ratio (95%CI)	P value
Variables			
MRD status (F1 ctDNA)	Positive vs negative	9.98 (4.73–21.07)	1.606E-09
Gender	Female vs male	0.85 (0.27–2.66)	0.782
Age		0.99 (0.95–1.04)	0.807
pCR status	Non-pCR vs pCR	3.41 (1.36–8.56)	0.009
Baseline tumor stage (TNM)	III-IVa vs II	0.99 (0.41–2.40)	0.979
Adjuvant immunotherapy	With vs without	0.51 (0.22–1.17)	0.111



Abbreviations: F1 ctDNA detected by tumor-informed personalized assay at 3–4 weeks post-surgery. pCR, pathological complete response.

(legend on next page)

factor for postoperative risk stratification in esophageal cancer. Current guidelines recommend adjuvant immunotherapy with nivolumab (a PD-1 inhibitor) for ESCC patients who do not achieve pCR after nCRT.[28,29] In line with this, non-pCR patients in this study exhibited worse DFS than pCR patients (p = 0.005; HR, 3.02; 95% CI, 1.35–6.77, Figure S5A), although there was no significant difference in OS (p = 0.055; HR, 2.34; 95% CI, 0.96–5.60, Figure S5B).

We next examined whether postoperative ctDNA could further stratify recurrence risk and guide adjuvant therapy decisions beyond conventional pathological response. Patients were categorized into four subgroups based on ctDNA status at F1 and pathological response. Those with positive ctDNA at F1 had worse DFS and OS than patients with negative ctDNA at F1, regardless of pCR or non-pCR (Log rank p for trend < 0.001; Figures S5C and S5D). Interestingly, DFS and OS were comparable between pCR and non-pCR patients who were ctDNA negative, raising the question whether this lack of difference may be partially due to the use of adjuvant immunotherapy in non-pCR patients.

We then stratified patients by ctDNA status at F1, which coincided with the timing prior to the initiation of adjuvant therapy. Among patients with positive ctDNA at F1 (n = 37), patients with pCR had better DFS compared to non-pCR patients who did not receive adjuvant immunotherapy (p = 0.019; HR, 0.31; 95% CI, 0.11–0.87, Figure 5A). Additionally, non-pCR patients receiving adjuvant immunotherapy had improved DFS compared to non-pCR patients not receiving adjuvant immunotherapy (p = 0.022; HR, 0.34; 95% CI, 0.13–0.89). While there was no significant difference in OS, a trend suggested an improved OS for non-pCR patients who received adjuvant immunotherapy than those not receiving it (Figure 5B). Conversely, among patients with negative ctDNA at F1 (n = 79), no significant differences were observed in DFS (p = 0.140, Figure 5C) or OS (p = 0.158, Figure 5D) between non-pCR patients with or without adjuvant immunotherapy and pCR patients. To address potential confounding from guideline changes over the study period (STAR Methods), we performed multivariable Cox regression analyses adjusting for clinical factors (age, gender, and baseline tumor stage) and guideline era effects. In patients with positive ctDNA post-surgery, non-pCR patients receiving adjuvant immunotherapy showed a significant improvement in prognosis compared to those not receiving adjuvant immunotherapy (adjusted HR 0.24, 95% CI 0.07–0.80, p = 0.021, Table S6). However, in patients with negative ctDNA post-surgery, adjuvant immunotherapy was not associated with a significant improvement in prognosis for non-pCR patients (p = 0.533, Table S6). These findings support the potential utility of post-surgical ctDNA to enhance risk stratification and guide personalized adjuvant immunotherapy decisions in ESCC.

Integrating ctDNA-based MRD into clinical management of esophageal cancer after neoadjuvant therapy

Based on the aforementioned results, a hypothetical scenario for integrating ctDNA-based systemic MRD assessment with clinical diagnostic modalities for LRD evaluation (Figure 6A) could be proposed. In ESCC patients following nCRT, prognostic outcomes can be categorized into four types based on their MRD and LRD status.

(1) Type 1: patients without residual disease at both locoregional and systemic sites; both LRD and ctDNA-based MRD would be negative after nCRT and remain negative post-surgery. These patients are expected to have good prognosis.
(2) Type 2: patients with residual disease confined to the locoregional tumor site would have positive results for both LRD and ctDNA-based MRD post-nCRT. However, after complete surgical resection, both LRD and ctDNA-based MRD would convert to negative. These patients are expected to have favorable outcomes.
(3) Type 3: residual disease is present beyond the locoregional site; LRD might be negative, but ctDNA-based MRD remains positive post-nCRT, and the positivity of ctDNA-based MRD would persist after radical surgery, indicating a higher risk of systemic recurrence.
(4) Type 4: patients with concurrent locoregional and systemic residual disease would have positive results for both LRD and ctDNA-based MRD post-nCRT; even after radical surgery, when LRD has been removed, ctDNA would remain positive, indicating persistent systemic residual disease and a poor prognosis.

This conceptual stratification highlights the complementary role of ctDNA-based MRD alongside conventional diagnostics in refining residual disease assessment and supporting personalized management in the post-nCRT setting of ESCC. Therefore, we propose a clinical pathway incorporating ctDNA-based MRD into post-neoadjuvant therapy management for ESCC patients (Figure 6B). At the first response evaluation post-nCRT, if either endoscopic biopsy or ctDNA is positive, immediate surgery is recommended. If both tests are negative, a second evaluation is scheduled 4–6 weeks later. At that point, patients with any positive endoscopic biopsy or ctDNA should proceed to surgery. Based on this strategy, the positive predictive value (PPV) for non-pCR would be 85.7% and 88.2% at the first and second response

Figure 4. Postoperative ctDNA status predicts prognosis after nCRT

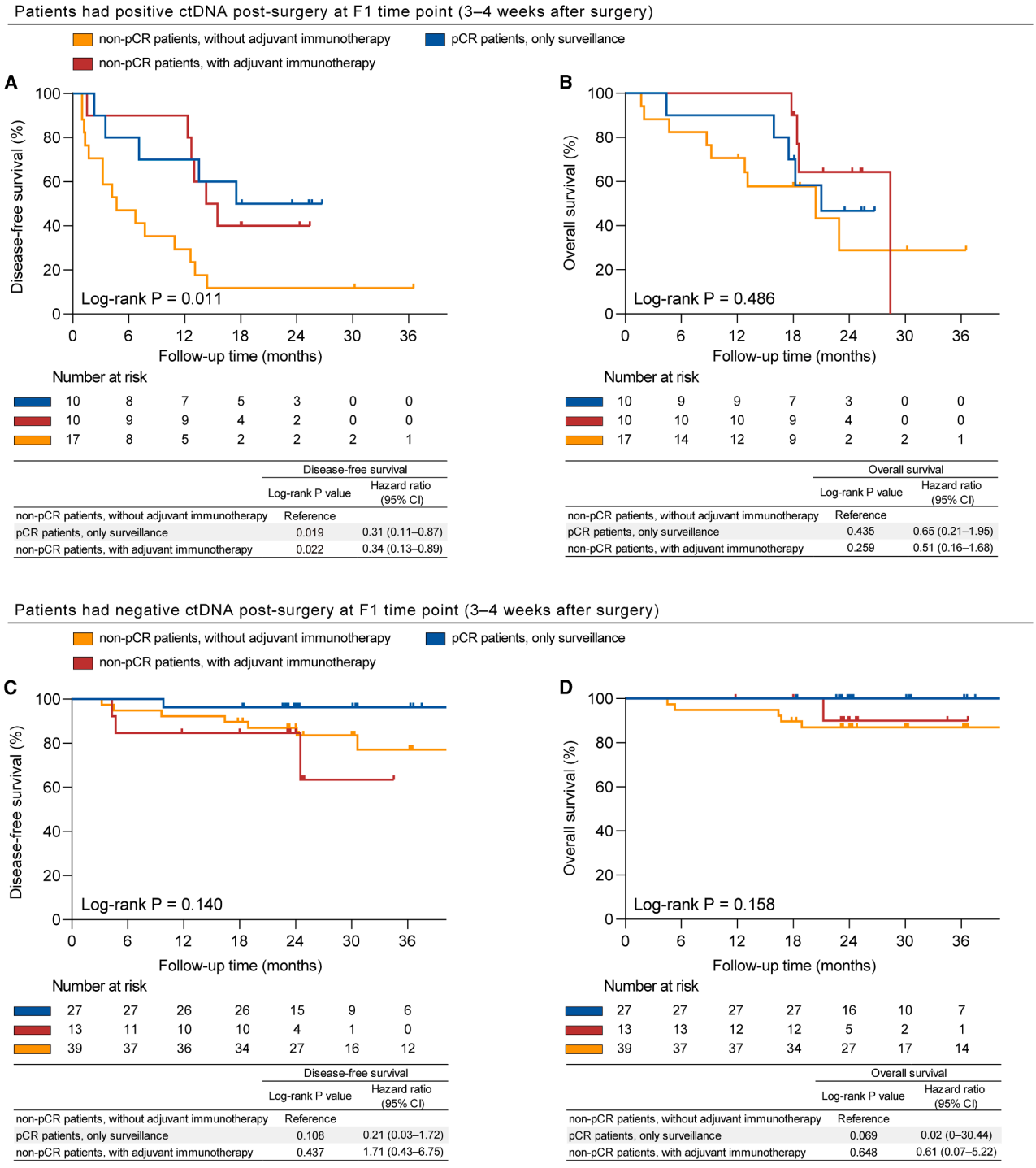
(A) Sankey plot showing the change in ctDNA-based MRD status from post-nCRT/pre-surgery (CRE-1/2) to post-surgery among patients with matched ctDNA results at all time points.

(B and C) Kaplan-Meier curves for (B) disease-free survival (DFS) and (C) overall survival (OS) stratified by ctDNA status changes from post-nCRT to post-surgery (F1): consistent negative, converted negative, converted positive, and consistent positive.

(D and E) Kaplan-Meier curves of (D) DFS and (E) OS between patients with positive and negative ctDNA at F1 time point.

(F) Forest plot of multivariable Cox regression analysis for DFS, showing adjusted hazard ratios for postoperative MRD status (F1 ctDNA), clinical factors, and adjuvant treatment.

Log rank test was used in (B)–(E).



	Disease-free survival	
	Log-rank P value	Hazard ratio (95% CI)
non-pCR patients, without adjuvant immunotherapy	Reference	
pCR patients, only surveillance	0.019	0.31 (0.11–0.87)
non-pCR patients, with adjuvant immunotherapy	0.022	0.34 (0.13–0.89)

	Overall survival	
	Log-rank P value	Hazard ratio (95% CI)
non-pCR patients, without adjuvant immunotherapy	Reference	
pCR patients, only surveillance	0.435	0.65 (0.21–1.95)
non-pCR patients, with adjuvant immunotherapy	0.259	0.51 (0.16–1.68)

	Disease-free survival	
	Log-rank P value	Hazard ratio (95% CI)
non-pCR patients, without adjuvant immunotherapy	Reference	
pCR patients, only surveillance	0.108	0.21 (0.03–1.72)
non-pCR patients, with adjuvant immunotherapy	0.437	1.71 (0.43–6.75)

	Overall survival	
	Log-rank P value	Hazard ratio (95% CI)
non-pCR patients, without adjuvant immunotherapy	Reference	
pCR patients, only surveillance	0.069	0.02 (0–30.44)
non-pCR patients, with adjuvant immunotherapy	0.648	0.61 (0.07–5.22)

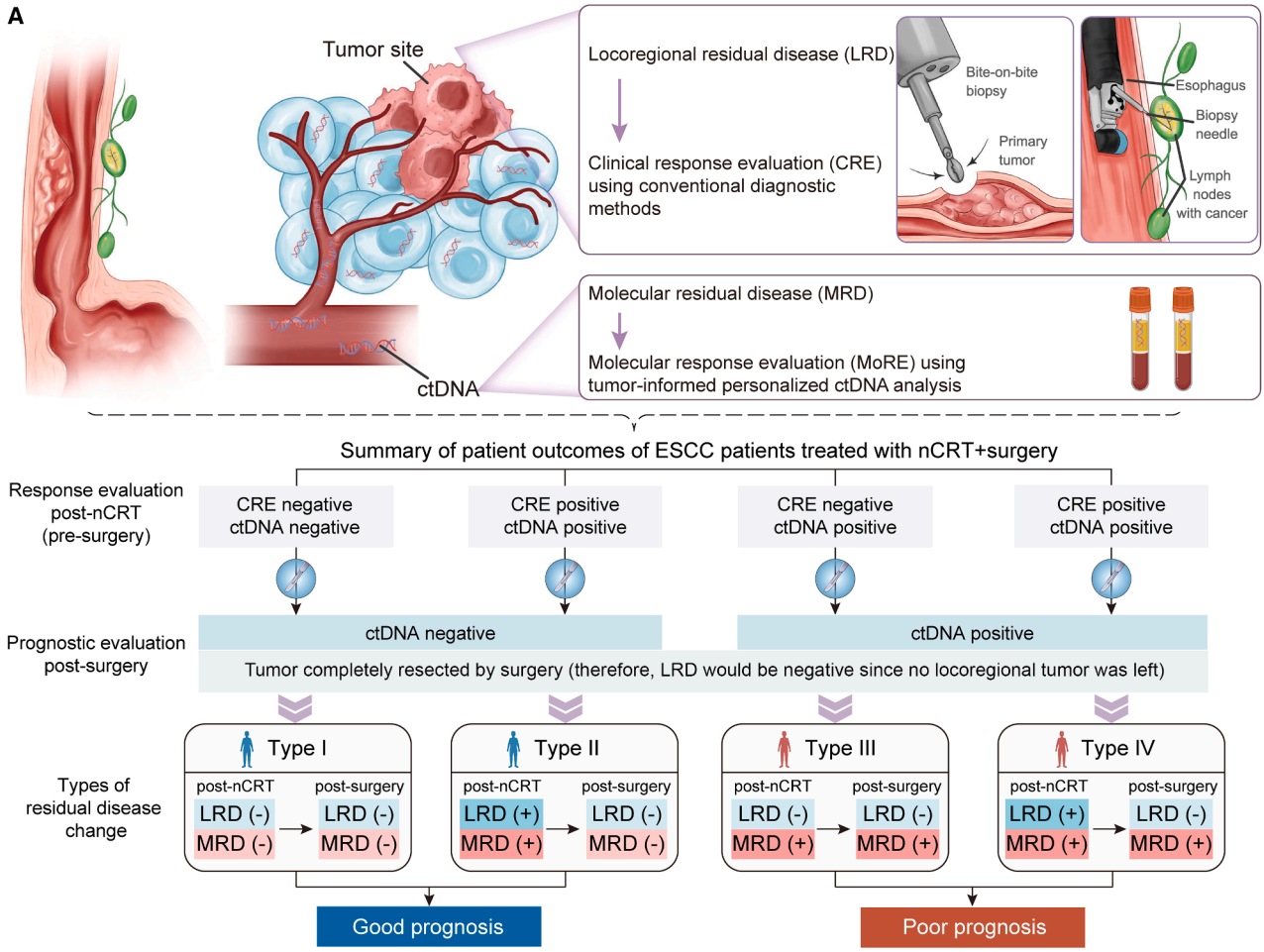
Figure 5. Postoperative ctDNA predicts benefit from adjuvant immunotherapy

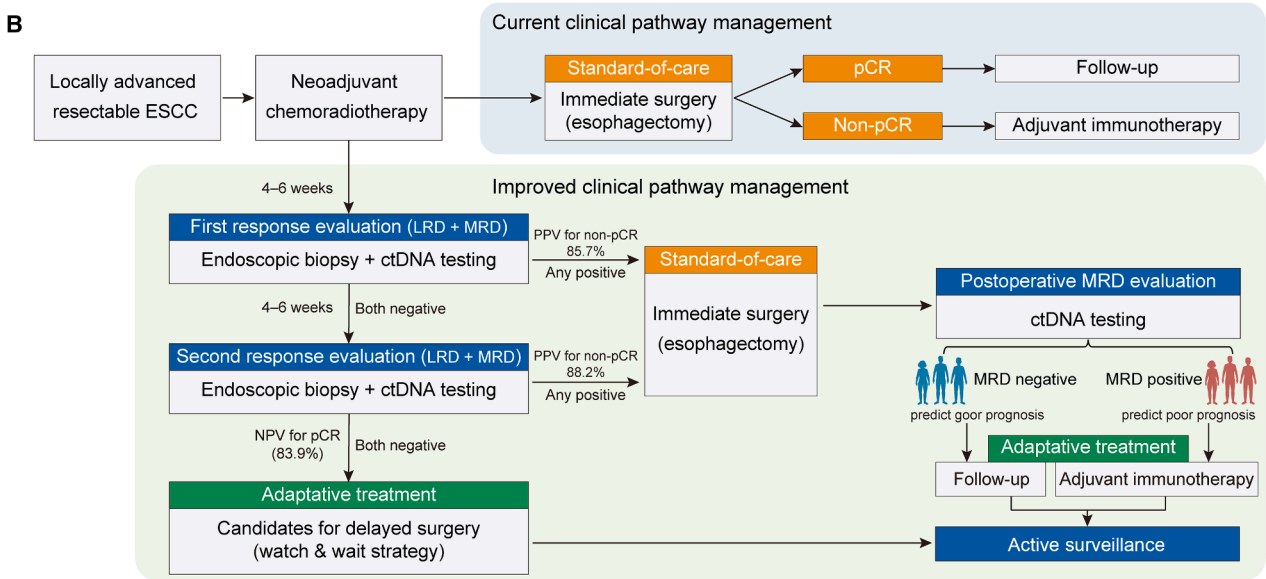
(A) Kaplan-Meier curves of disease-free survival (DFS) for patients with ctDNA positivity at F1 time point, stratified by three subgroups: pathological complete response (pCR) receiving surveillance only, non-pCR with adjuvant immunotherapy, and non-pCR without adjuvant immunotherapy. See also Figure S5 and Table S6.

(B) Kaplan-Meier curves of overall survival (OS) for the same patient groups as in (A).

(C and D) Kaplan-Meier curves of (C) DFS and (D) OS for patients with ctDNA negativity at F1 time point, stratified by the same three subgroups as in (A).

Log rank test was used in (A)–(D); hazard ratio was calculated using Cox regression.





(legend on next page)

evaluation, respectively. Patients who remain negative by both endoscopic biopsy and ctDNA may be eligible for a "Watch and Wait" strategy but should be regularly monitored with ctDNA testing since the negative predictive value for pCR would be 83.9%. For patients who undergo surgery (current standard of care) after nCRT, postoperative ctDNA may further inform adjuvant treatment decisions. Patients with positive ctDNA post-surgery are at higher risk for recurrence and may benefit from adjuvant therapy (e.g., immunotherapy), while those with negative ctDNA post-surgery may only need standard follow-up care.

DISCUSSION

In this prospective study of 132 patients with locally advanced resectable ESCC treated with nCRT followed by response diagnostic tests and surgery, we provide evidence that integrating molecular diagnostics (ctDNA-based MRD detection) with conventional clinical diagnostics improves the accuracy of residual disease detection after nCRT. Moreover, our findings support postoperative ctDNA status as a prognostic marker for recurrence risk and a potential predictive biomarker for benefit from adjuvant therapy. These findings support the incorporation of ctDNA-based MRD evaluation into the current neoadjuvant and perioperative management paradigm to enable adaptive and individualized treatment for ESCC patients.

The adoption of organ-sparing strategies as an alternative to standard esophagectomy for patients achieving cCR post-neoadjuvant therapy requires a highly accurate diagnostic approach for residual disease detection. While the previous preSANO trial established the utility of CRE with endoscopic bite-on-bite biopsy and EUS-FNA for detecting major residual disease (>10% residual cancer), the conventional clinical diagnostics have notable limitations, including the inability to assess systemic MRD, using invasive diagnostic procedures, and technical difficulties in suspected lymph node aspirations.[26] Importantly, from an oncological safety standpoint, even 1%–10% residual cancer should ideally be identified, as true pCR is a more appropriate selection criterion for safe organ preservation than MPR. However, previous studies demonstrated that conventional clinical diagnostics exhibited substantial false-negative rates in residual disease detection, misclassifying 23% of non-pCR patients as cCR in the European preSANO cohort[26] and 18.3% in the Asian preSINO cohort.[19] These limitations highlight the need for a more sensitive diagnostic approach to capture MRD and ensure oncological safety for organ preservation candidates.

ctDNA has emerged as a transformative biomarker, with recent studies extending its role beyond prognosis to predicting treatment response.[11,30–32] However, its utility in identifying cCR post-nCRT in esophageal cancer remains unclear. A key question is how to effectively integrate ctDNA-based MoRE with conventional CRE strategies to improve diagnostic accuracy. Notably, the clinical validity of ctDNA depends on assay design. A retrospective study involving 31 esophageal cancer patients reported that a small fixed-panel ctDNA assay was ineffective for residual disease detection after nCRT.[23] More recent studies highlight the superiority of tumor-informed personalized assay over tumor-naive fixed-panel assays due to its greater sensitivity and ability to address tumor heterogeneity.[13,33] In our study, we comprehensively compared the sensitivity of three ctDNA assays, including a tumor-informed personalized panel, a tumor-naive fixed panel, and a tumor-informed fixed panel, alongside standard clinical diagnostic methods, in detecting residual disease in ESCC treated with nCRT. The tumor-informed personalized panel demonstrated the highest positivity and the lowest limit of detection, both before and after treatment, supporting its superior sensitivity.[13] Notably, our findings reveal a higher baseline ctDNA-positive rate (99.2%) by personalized assay compared to rates reported in previous studies (60%–70%)[21–25] using fixed-panel assays. These custom fixed panels covered only 2.4%–10.0% of the patient-specific variant sites tracked by our tumor-informed personalized panel. Furthermore, among the variants selected for tracking in our personalized assay, 99.5% were unique to individual patients, reflecting minimal inter-patient overlap. These findings highlight the biological heterogeneity of ESCC and provide a rationale for the superior sensitivity of tumor-informed personalized assays in detecting MRD. As an illustrative example, in the small subset of patients with clinical stage IVa disease, which is commonly associated with a greater tumor burden, the tumor-naive fixed panel showed a low ctDNA detection rate (22.2%). This may reflect both the small sample size and the limited ability of fixed panels to capture patient-specific mutations, particularly in advanced tumors with high genomic heterogeneity. In contrast, the tumor-informed personalized assay detected ctDNA in all stage IVa cases and maintained high detection rates across other stages, suggesting its robustness under biologically and technically challenging conditions. Importantly, our study showed that combining ctDNA with conventional clinical diagnostics reduced the false-negative rate for detecting any residual disease by around 13%, helping prevent misclassification of non-pCR patients as cCR and allowing timely interventions.

Our study revealed that post-nCRT ctDNA assessment could identify patients at risk of postoperative distant metastasis, even among those classified as cCR by conventional CRE. This highlights the value of ctDNA as a molecular supplement to conventional clinical diagnostics, which fails to identify high-risk patients with occult systemic residual disease. The SANO trial reported high distant recurrence rates in cCR patients assessed by conventional CRE methods, regardless of undergoing surgery (34%) or active surveillance (43%).[9,10] These findings highlight the limitations of conventional diagnostic methods, which primarily focus on locoregional tumor assessment (e.g., endoscopy) or

Figure 6. Proposed strategy for integrating ctDNA testing into post-neoadjuvant management of esophageal cancer

(A) Summary of residual disease evaluation and outcome stratification for ESCC patients receiving neoadjuvant therapy and surgery. The diagram illustrates four types of residual disease changes based on locoregional residual disease and molecular residual disease status, with corresponding prognostic implications.
(B) Graphical illustration of a proposed improved clinical pathway incorporating ctDNA testing for response evaluation and postoperative risk stratification. The upper panel shows the current standard-of-care pathway, while the lower panel outlines an adaptive management strategy using ctDNA-based molecular response evaluation to guide adaptive treatment decisions.

macroscopic metastasis identification (e.g., PET-CT imaging), and emphasize the need for systemic MRD evaluation through methods such as ctDNA analysis. Importantly, it should be noted that salvage surgery will not be a feasible option if distant dissemination occurs during active surveillance for patients classified as cCR, leading to the failure of "Watch and Wait" strategy. In this context, our findings support the use of ctDNA to improve identification of cCR patients truly suitable for active surveillance. Among patients evaluated as cCR by conventional CRE (biopsy and EUS-FNA), the distant recurrence rate within 1 year was significantly lower (2.7%) in those who were ctDNA negative post-nCRT compared to those who had positive ctDNA post-nCRT (19.0%).[19] This indicates that ctDNA negativity after nCRT may serve as a strong indicator for selecting patients at low risk of distant recurrence who could benefit from non-surgical management. While integrating ctDNA with conventional diagnostics reduced false-negative rates for residual disease detection, it increased the classification of pCR patients as non-clinical complete responders. Notably, the 11 pCR patients with positive ctDNA post-nCRT exhibited significantly higher postoperative distant metastasis rates (4/11, 36.4%), indicating possible occult systemic residual disease. However, some patients remained recurrence free, highlighting the need for specificity optimization to reduce overtreatment risk. Therefore, combining ctDNA analysis with imaging (e.g., PET-CT radiomics) or other biomarkers is warranted in future studies to robustly differentiate "true" from possibly "false" positive results. Such integrated approaches may help optimize treatment intensity while preserving oncological safety.

Currently, there is a lack of effective methods for postoperative risk stratification in patients with esophageal cancer after neoadjuvant therapy. Despite the use of combined neoadjuvant therapy and surgery, recurrence rates remain high for esophageal cancer.[34–36] The CheckMate 577 trial demonstrated that adjuvant immunotherapy reduces recurrence and improves DFS.[28] However, apart from non-pCR, decisions regarding adjuvant immunotherapy often rely on clinical judgment, which may lead to overtreatment or undertreatment in some patients. Given that ctDNA testing has been shown to predict the recurrence risk, it may serve as a valuable tool to guide adjuvant therapy in solid tumors. The GALAXY study showed that significant benefit from adjuvant chemotherapy was observed in colorectal cancer patients with post-surgical ctDNA positivity but not in those with ctDNA negativity, suggesting that ctDNA testing could guide adjuvant treatment decisions.[11] Similarly, the DYNAMIC study demonstrated that a ctDNA-guided strategy in stage II colon cancer reduced the use of adjuvant chemotherapy without compromising survival.[15] Our study provides supportive data for the use of ctDNA analysis in postoperative risk stratification in ESCC. Specifically, we show that postoperative ctDNA positivity may serve as a useful biomarker for identifying patients who could benefit from adjuvant immunotherapy for ESCC undergoing nCRT. Importantly, we observed that even pCR patients—those theoretically cured—had worse survival outcomes when ctDNA was positive after surgery, indicating a potential need for adjuvant therapy. Additionally, non-pCR patients with positive ctDNA post-surgery benefited significantly from adjuvant immunotherapy, while non-pCR patients with negative ctDNA post-surgery did not. This suggests that ctDNA-negative patients might not require adjuvant immunotherapy after surgery, potentially reducing unnecessary treatment-related side effects and economic burden.

In conclusion, this prospective cohort study from the preSINO trial evaluated the clinical value of ctDNA in guiding perioperative decision-making after neoadjuvant therapy by assessing the ctDNA-based MRD status at key perioperative time points (baseline, post-nCRT, and post-surgery). We demonstrated the potential of personalized ctDNA analysis in identifying residual disease post-neoadjuvant treatment, thereby supporting organ-sparing strategies. Additionally, postoperative ctDNA positivity stratified recurrence risk and informed adjuvant therapy decisions. Our findings highlight the transformative potential of ctDNA in enabling adaptive, individualized treatment strategies. Importantly, ctDNA is optimally applied in conjunction with standard response evaluation and surveillance diagnostics, offering a complementary approach that enhances sensitivity and reduces the risk of undertreatment. This study provides important evidence supporting a shift from empiric treatment paradigms toward molecularly guided precision oncology in esophageal cancer management.

Limitations of the study

This study had several limitations. First, our exploratory study was observational in nature and lacked intervention based on ctDNA detection. Our findings demonstrate the potential utility of ctDNA-guided adaptive management in ESCC. However, the absence of protocol-directed interventions, non-randomized design, and limited cohort size necessitate cautious interpretation of our work. Larger intervention studies and randomized trials are required to validate our proof-of-concept findings. We have initiated a clinical trial (NCT06861894) to validate organ-sparing selection based on ctDNA analysis combined with conventional clinical diagnostics. Second, our study only included ESCC, and the applicability of ctDNA for residual disease detection following neoadjuvant therapy in esophageal adenocarcinoma (EAC) requires further investigation. Nevertheless, the demonstrated utility of tumor-informed personalized ctDNA assay in multiple cancers with different genetic backgrounds and biology[13,31,37] suggests its potential applicability in EAC. A recent small-scale study in 23 EAC patients achieving pCR revealed that post-surgical ctDNA positivity correlated with increased recurrence risk, aligning with our ESCC observations. Further large-scale studies are needed to confirm the utility of ctDNA in EAC. Third, while our study highlights the potential clinical value of tumor-informed personalized ctDNA testing, a major limitation is the higher cost, which may hinder real-world implementation. In the United States, clinical adoption of personalized ctDNA (e.g., Signatera) has been facilitated by reimbursement from the Centers for Medicare & Medicaid Services for indications including adjuvant and/or recurrence monitoring in colorectal cancer,[38] bladder cancer,[39] breast cancer,[31] and ovarian cancer.[40] However, in countries where such testing is not yet included in public reimbursement programs, such as most regions in Europe and China, the cost may limit routine adoption. This is likely due to insufficient region-specific clinical evidence to support cost-effective evaluations and reimbursement decisions. Taking the perioperative management of esophageal cancer as an example, this technology may offset expenses by reducing overtreatment (e.g., unnecessary surgery) in

low-risk individuals and preventing costly metastatic progression through early adjuvant intervention in high-risk patients, thus potentially reducing associated healthcare expenses. Continued technological improvements and the accumulation of clinical data from diverse populations, including European[41,42] and Asian[11,13,43] cohorts, will further support clinical feasibility and cost reduction. To enable broader adoption, future research should prioritize technology assessments, cost-effectiveness, and optimized workflows that balance sensitivity, feasibility, and affordability. Fourth, non-genetic-based liquid biopsy such as epigenomic- and fragmentomic-based assays have also emerged as promising tools for MRD detection.[44–46] These tumor-naive assays offer the advantage of not requiring matched tumor tissues to detect MRD and have demonstrated promising sensitivity in some settings. However, our study did not compare the performance of the tumor-informed personalized assay with the aforementioned non-genetic assays; future head-to-head comparisons are warranted to evaluate their relative performance. These methods may serve as complementary tools to tumor-informed assays, depending on clinical context and resource availability.[47] Additionally, no gender-stratified analyses were conducted.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Zhigang Li (zhigang.li@shsmu.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive[48] in the National Genomics Data Center[49] (GSA-Human: HRA010802 (https://ngdc.cncb.ac.cn/gsa-human/browse/HRA010802)). The data will be accessible for scientific research upon reasonable request complying with the law due to human patient privacy concerns. Requests for access to the patient-level data from this study can be submitted via email to the lead contact with a detailed proposal for approval.
- This paper does not report any original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

ACKNOWLEDGMENTS

The authors thank the patients and their families, physicians, physician assistants, and nurses who participated in this study. We also appreciate the contribution of Chunyu Zhang, Mingjun Zhu, Songan Chen, Jianxing Xiang, and Pei Gu for their assistance with sample collection and reservation. This work was supported by National Clinical Key Specialty Construction Project (10000015Z155080000004), National Natural Science Foundation of China (82320108016 and 82403831), Program of Shanghai Academic/Technology Research Leader (22XD1402900), Shanghai Hospital Development Center (SHDC2025CCS023 and SHDC22024225), and National Key Research and Development Program of China (2021YFC2501005). The funding sources had no role in the preparation of this manuscript.

AUTHOR CONTRIBUTIONS

Conceptualization, Z. Liu and Z. Li; methodology, Z. Liu, G.W., P.C., X.F., J.Y., Z.Z., X.G., Y.C., B.M., J.J.B.v.L., B.P.L.W., S.L., S.C., and Z. Li; investigation, Z. Liu, C.L., Y.Y., Y.S., H.Z., and J.L.; data curation, Z. Liu, C.L., Y.Y., Y.S., and H.Z.; formal analysis, Z. Liu, G.W., P.C., X.F., J.Y., and S.C.; writing – original draft, Z. Liu and Z. Li; review and editing, all the authors; resources, C.L., S.C., and Z. Li; funding acquisition and supervision, Z. Li.

DECLARATION OF INTERESTS

X.G. has received a personal research grant from the Nijbakker-Morra Foundation. G.W., P.C., X.F., J.Y., Z.Z., and S.C. are employees of Burning Rock Biotech.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - Study design and participants
- METHOD DETAILS
  - Study outcomes and objectives
  - Postoperative adjuvant immunotherapy
  - Postoperative follow-up
  - Sample collection, processing and sequencing
  - Personalized ctDNA assay for MRD detection
  - Somatic mutation caller for fixed targeted panel
- QUANTIFICATION AND STATISTICAL ANALYSIS
- ADDITIONAL RESOURCES

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrm.2025.102334.

Received: March 20, 2025
Revised: July 4, 2025
Accepted: August 12, 2025
Published: September 5, 2025

REFERENCES

1. Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R.L., Soerjomataram, I., and Jemal, A. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. *74*, 229–263. https://doi.org/10.3322/caac.21834.
2. Abnet, C.C., Arnold, M., and Wei, W.Q. (2018). Epidemiology of Esophageal Squamous Cell Carcinoma. Gastroenterology *154*, 360–373. https://doi.org/10.1053/j.gastro.2017.08.023.
3. van Hagen, P., Hulshof, M.C.C.M., van Lanschot, J.J.B., Steyerberg, E.W., van Berge Henegouwen, M.I., Wijnhoven, B.P.L., Richel, D.J., Nieuwenhuijzen, G.A.P., Hospers, G.A.P., Bonenkamp, J.J., et al. (2012). Preoperative chemoradiotherapy for esophageal or junctional cancer. N. Engl. J. Med. *366*, 2074–2084. https://doi.org/10.1056/NEJMoa1112088.
4. Yang, H., Liu, H., Chen, Y., Zhu, C., Fang, W., Yu, Z., Mao, W., Xiang, J., Han, Y., Chen, Z., et al. (2018). Neoadjuvant Chemoradiotherapy Followed by Surgery Versus Surgery Alone for Locally Advanced Squamous Cell Carcinoma of the Esophagus (NEOCRTEC5010): A Phase III Multicenter, Randomized, Open-Label Clinical Trial. J. Clin. Oncol. *36*, 2796–2803. https://doi.org/10.1200/JCO.2018.79.1483.
5. Shah, M.A., Altorki, N., Patel, P., Harrison, S., Bass, A., and Abrams, J.A. (2023). Improving outcomes in patients with oesophageal cancer. Nat. Rev. Clin. Oncol. *20*, 390–407. https://doi.org/10.1038/s41571-023-00757-y.
6. van der Wilk, B.J., Eyck, B.M., Hofstetter, W.L., Ajani, J.A., Piessen, G., Castoro, C., Alfieri, R., Kim, J.H., Kim, S.B., Furlong, H., et al. (2022). Chemoradiotherapy Followed by Active Surveillance Versus Standard Esophagectomy for Esophageal Cancer: A Systematic Review and Individual Patient Data Meta-analysis. Ann. Surg. *275*, 467–476. https://doi.org/10.1097/SLA.0000000000004930.

7. Noordman, B.J., Verdam, M.G.E., Lagarde, S.M., Shapiro, J., Hulshof, M.C.C.M., van Berge Henegouwen, M.I., Wijnhoven, B.P.L., Nieuwenhuijzen, G.A.P., Bonenkamp, J.J., Cuesta, M.A., et al. (2018). Impact of neoadjuvant chemoradiotherapy on health-related quality of life in long-term survivors of esophageal or junctional cancer: results from the randomized CROSS trial. Ann. Oncol. *29*, 445–451. https://doi.org/10.1093/annonc/mdx726.

8. O'Connell, L., Coleman, M., Kharyntiuk, N., and Walsh, T.N. (2019). Quality of life in patients with upper GI malignancies managed by a strategy of chemoradiotherapy alone versus surgery. Surg. Oncol. *30*, 33–39. https://doi.org/10.1016/j.suronc.2019.05.021.

9. van der Wilk, B.J., Eyck, B.M., Wijnhoven, B.P.L., Lagarde, S.M., Rosman, C., Noordman, B.J., Valkema, M.J., Coene, P.P., Dekker, J.W., Hartgrink, H., et al. (2023). LBA75 Neoadjuvant chemoradiotherapy followed by surgery versus active surveillance for oesophageal cancer (SANO-trial): A phase-III stepped-wedge cluster randomised trial. Ann. Oncol. *34*, S1317. https://doi.org/10.1016/j.annonc.2023.10.076.

10. van der Wilk, B.J., Eyck, B.M., Wijnhoven, B.P.L., Lagarde, S.M., Rosman, C., Noordman, B.J., Valkema, M.J., Bisseling, T.M., Coene, P.-P.L.O., van Det, M.J., et al. (2025). Neoadjuvant chemoradiotherapy followed by active surveillance versus standard surgery for oesophageal cancer (SANO trial): a multicentre, stepped-wedge, cluster-randomised, non-inferiority, phase 3 trial. Lancet Oncol. *26*, 425–436. https://doi.org/10.1016/S1470-2045(25)00027-0.

11. Kotani, D., Oki, E., Nakamura, Y., Yukami, H., Mishima, S., Bando, H., Shirasu, H., Yamazaki, K., Watanabe, J., Kotaka, M., et al. (2023). Molecular residual disease and efficacy of adjuvant chemotherapy in patients with colorectal cancer. Nat. Med. *29*, 127–134. https://doi.org/10.1038/s41591-022-02115-4.

12. Moding, E.J., Nabet, B.Y., Alizadeh, A.A., and Diehn, M. (2021). Detecting Liquid Remnants of Solid Tumors: Circulating Tumor DNA Minimal Residual Disease. Cancer Discov. *11*, 2968–2986. https://doi.org/10.1158/2159-8290.CD-21-0634.

13. Chen, K., Yang, F., Shen, H., Wang, C., Li, X., Chervova, O., Wu, S., Qiu, F., Peng, D., Zhu, X., et al. (2023). Individualized tumor-informed circulating tumor DNA analysis for postoperative monitoring of non-small cell lung cancer. Cancer Cell *41*, 1749–1762.e6. https://doi.org/10.1016/j.ccell.2023.08.010.

14. Abbosh, C., Frankell, A.M., Harrison, T., Kisistok, J., Garnett, A., Johnson, L., Veeriah, S., Moreau, M., Chesh, A., Chaunzwa, T.L., et al. (2023). Tracking early lung cancer metastatic dissemination in TRACERx using ctDNA. Nature *616*, 553–562. https://doi.org/10.1038/s41586-023-05776-4.

15. Tie, J., Cohen, J.D., Lahouel, K., Lo, S.N., Wang, Y., Kosmider, S., Wong, R., Shapiro, J., Lee, M., Harris, S., et al. (2022). Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer. N. Engl. J. Med. *386*, 2261–2272. https://doi.org/10.1056/NEJMoa2200075.

16. Provencio, M., Serna-Blasco, R., Nadal, E., Insa, A., García-Campelo, M.R., Casal Rubio, J., Dómine, M., Majem, M., Rodríguez-Abreu, D., Martínez-Martí, A., et al. (2022). Overall Survival and Biomarker Analysis of Neoadjuvant Nivolumab Plus Chemotherapy in Operable Stage IIIA Non-Small-Cell Lung Cancer (NADIM phase II trial). J. Clin. Oncol. *40*, 2924–2933. https://doi.org/10.1200/JCO.21.02660.

17. Forde, P.M., Spicer, J., Lu, S., Provencio, M., Mitsudomi, T., Awad, M.M., Felip, E., Broderick, S.R., Brahmer, J.R., Swanson, S.J., et al. (2022). Neoadjuvant Nivolumab plus Chemotherapy in Resectable Lung Cancer. N. Engl. J. Med. *386*, 1973–1985. https://doi.org/10.1056/NEJMoa2202170.

18. Magbanua, M.J.M., Swigart, L.B., Wu, H.T., Hirst, G.L., Yau, C., Wolf, D.M., Tin, A., Salari, R., Shchegrova, S., Pawar, H., et al. (2021). Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. Ann. Oncol. *32*, 229–239. https://doi.org/10.1016/j.annonc.2020.11.007.

19. Yang, Y., Liu, Z., Wong, I., Gao, X., Zhang, H., Liu, J., Eyck, B.M., Shao, J., Han, Y., van der Wilk, B.J., et al. (2025). Detecting residual disease after neoadjuvant chemoradiotherapy for oesophageal squamous cell carcinoma: The prospective multicentre preSINO trial. Br. J. Surg. *112*, znaf004. https://doi.org/10.1093/bjs/znaf004.

20. Deveson, I.W., Gong, B., Lai, K., LoCoco, J.S., Richmond, T.A., Schageman, J., Zhang, Z., Novoradovskaya, N., Willey, J.C., Jones, W., et al. (2021). Evaluating the analytical validity of circulating tumor DNA sequencing assays for precision oncology. Nat. Biotechnol. *39*, 1115–1128. https://doi.org/10.1038/s41587-021-00857-z.

21. Wang, X., Yu, N., Cheng, G., Zhang, T., Wang, J., Deng, L., Li, J., Zhao, X., Xu, Y., Yang, P., et al. (2022). Prognostic value of circulating tumour DNA during post-radiotherapy surveillance in locally advanced esophageal squamous cell carcinoma. Clin. Transl. Med. *12*, e1116. https://doi.org/10.1002/ctm2.1116.

22. Ng, H.Y., Ko, J.M.Y., Lam, K.O., Kwong, D.L.W., Lo, A.W.I., Wong, I.Y.H., Wong, C.L.Y., Chan, S.Y., Chan, K.K., Law, T.T., et al. (2023). Circulating Tumor DNA Dynamics as Prognostic Markers in Locally Advanced and Metastatic Esophageal Squamous Cell Carcinoma. JAMA Surg. *158*, 1141–1150. https://doi.org/10.1001/jamasurg.2023.4395.

23. Eyck, B.M., Jansen, M.P., Noordman, B.J., Atmodimedjo, P.N., van der Wilk, B.J., Martens, J.W., Helmijr, J.A., Beaufort, C.M., Mostert, B., Doukas, M., et al. (2023). Detection of circulating tumour DNA after neoadjuvant chemoradiotherapy in patients with locally advanced oesophageal cancer. J. Pathol. *259*, 35–45. https://doi.org/10.1002/path.6016.

24. Azad, T.D., Chaudhuri, A.A., Fang, P., Qiao, Y., Esfahani, M.S., Chabon, J.J., Hamilton, E.G., Yang, Y.D., Lovejoy, A., Newman, A.M., et al. (2020). Circulating Tumor DNA Analysis for Detection of Minimal Residual Disease After Chemoradiotherapy for Localized Esophageal Cancer. Gastroenterology *158*, 494–505.e6. https://doi.org/10.1053/j.gastro.2019.10.039.

25. Chen, B., Liu, S., Zhu, Y., Wang, R., Cheng, X., Chen, B., Dragomir, M.P., Zhang, Y., Hu, Y., Liu, M., et al. (2024). Predictive role of ctDNA in esophageal squamous cell carcinoma receiving definitive chemoradiotherapy combined with toripalimab. Nat. Commun. *15*, 1919. https://doi.org/10.1038/s41467-024-46307-7.

26. Noordman, B.J., Spaander, M.C.W., Valkema, R., Wijnhoven, B.P.L., van Berge Henegouwen, M.I., Shapiro, J., Biermann, K., van der Gaast, A., van Hillegersberg, R., Hulshof, M.C.C.M., et al. (2018). Detection of residual disease after neoadjuvant chemoradiotherapy for oesophageal cancer (preSANO): a prospective multicentre, diagnostic cohort study. Lancet Oncol. *19*, 965–974. https://doi.org/10.1016/S1470-2045(18)30201-8.

27. Nilsson, K., Klevebro, F., Sunde, B., Rouvelas, I., Lindblad, M., Szabo, E., Halldestam, I., Smedh, U., Wallner, B., Johansson, J., et al. (2023). Oncological outcomes of standard versus prolonged time to surgery after neoadjuvant chemoradiotherapy for oesophageal cancer in the multicentre, randomised, controlled NeoRes II trial. Ann. Oncol. *34*, 1015–1024. https://doi.org/10.1016/j.annonc.2023.08.010.

28. Kelly, R.J., Ajani, J.A., Kuzdzal, J., Zander, T., Van Cutsem, E., Piessen, G., Mendez, G., Feliciano, J., Motoyama, S., Lièvre, A., et al. (2021). Adjuvant Nivolumab in Resected Esophageal or Gastroesophageal Junction Cancer. N. Engl. J. Med. *384*, 1191–1203. https://doi.org/10.1056/NEJMoa2032125.

29. Esophageal and Esophagogastric Junction Cancers, Version 1.2025, NCCN Clinical Practice Guidelines in Oncology. https://www.nccn.org/guidelines/guidelines-detail?category=1&id=1433.

30. Kelly, R.J., Landon, B.V., Zaidi, A.H., Singh, D., Canzoniero, J.V., Balan, A., Hales, R.K., Voong, K.R., Battafarano, R.J., Jobe, B.A., et al. (2024). Neoadjuvant nivolumab or nivolumab plus LAG-3 inhibitor relatlimab in resectable esophageal/gastroesophageal junction cancer: a phase Ib trial and ctDNA analyses. Nat. Med. *30*, 1023–1034. https://doi.org/10.1038/s41591-024-02877-z.

31. Magbanua, M.J.M., Brown Swigart, L., Ahmed, Z., Sayaman, R.W., Renner, D., Kalashnikova, E., Hirst, G.L., Yau, C., Wolf, D.M., Li, W., et al. (2023). Clinical significance and biology of circulating tumor DNA in high-risk early-stage HER2-negative breast cancer receiving neoadjuvant chemotherapy. Cancer Cell *41*, 1091–1102.e4. https://doi.org/10.1016/j.ccell.2023.04.008.

32. Pan, Y., Zhang, J.T., Gao, X., Chen, Z.Y., Yan, B., Tan, P.X., Yang, X.R., Gao, W., Gong, Y., Tian, Z., et al. (2023). Dynamic circulating tumor DNA during chemoradiotherapy predicts clinical outcomes for locally advanced non-small cell lung cancer patients. Cancer Cell *41*, 1763–1773.e4. https://doi.org/10.1016/j.ccell.2023.09.007.

33. Santonja, A., Cooper, W.N., Eldridge, M.D., Edwards, P.A.W., Morris, J.A., Edwards, A.R., Zhao, H., Heider, K., Couturier, D.L., Vijayaraghavan, A., et al. (2023). Comparison of tumor-informed and tumor-naive sequencing assays for ctDNA detection in breast cancer. EMBO Mol. Med. *15*, e16505. https://doi.org/10.15252/emmm.202216505.

34. Blum Murphy, M., Xiao, L., Patel, V.R., Maru, D.M., Correa, A.M., G Amlashi, F., Liao, Z., Komaki, R., Lin, S.H., Skinner, H.D., et al. (2017). Pathological complete response in patients with esophageal cancer after the trimodality approach: The association with baseline variables and survival-The University of Texas MD Anderson Cancer Center experience. Cancer *123*, 4106–4113. https://doi.org/10.1002/cncr.30953.

35. Eyck, B.M., van Lanschot, J.J.B., Hulshof, M.C.C.M., van der Wilk, B.J., Shapiro, J., van Hagen, P., van Berge Henegouwen, M.I., Wijnhoven, B. P.L., van Laarhoven, H.W.M., Nieuwenhuijzen, G.A.P., et al. (2021). Ten-Year Outcome of Neoadjuvant Chemoradiotherapy Plus Surgery for Esophageal Cancer: The Randomized Controlled CROSS Trial. J. Clin. Oncol. *39*, 1995–2004. https://doi.org/10.1200/JCO.20.03614.

36. Zhong, J., Fang, S., Chen, R., Yuan, J., Xie, X., Lin, T., Liu, M., Liu, Q., and Fu, J. (2024). The patterns and risk factors for relapse in oesophageal squamous cell cancers that achieve pathological complete response to neoadjuvant chemoradiotherapy. Eur. J. Cardio. Thorac. Surg. *65*, ezae207. https://doi.org/10.1093/ejcts/ezae207.

37. Martinez-Castedo, B., Camblor, D.G., Martin-Arana, J., Carbonell-Asins, J.A., Garcia-Mico, B., Gambardella, V., Huerta, M., Rosello, S., Roda, D., Gimeno-Valiente, F., et al. (2025). Minimal residual disease in colorectal cancer. Tumor-informed versus tumor-agnostic approaches: unraveling the optimal strategy. Ann. Oncol. *36*, 263–276. https://doi.org/10.1016/j.annonc.2024.12.006.

38. Reinert, T., Henriksen, T.V., Christensen, E., Sharma, S., Salari, R., Sethi, H., Knudsen, M., Nordentoft, I., Wu, H.T., Tin, A.S., et al. (2019). Analysis of Plasma Cell-Free DNA by Ultradeep Sequencing in Patients With Stages I to III Colorectal Cancer. JAMA Oncol. *5*, 1124–1131. https://doi.org/10.1001/jamaoncol.2019.0528.

39. Christensen, E., Birkenkamp-Demtröder, K., Sethi, H., Shchegrova, S., Salari, R., Nordentoft, I., Wu, H.T., Knudsen, M., Lamy, P., Lindskrog, S.V., et al. (2019). Early Detection of Metastatic Relapse and Monitoring of Therapeutic Efficacy by Ultra-Deep Sequencing of Plasma Cell-Free DNA in Patients With Urothelial Bladder Carcinoma. J. Clin. Oncol. *37*, 1547–1557. https://doi.org/10.1200/JCO.18.02052.

40. Hou, J.Y., Chapman, J.S., Kalashnikova, E., Pierson, W., Smith-McCune, K., Pineda, G., Vattakalam, R.M., Ross, A., Mills, M., Suarez, C.J., et al. (2022). Circulating tumor DNA monitoring for early recurrence detection in epithelial ovarian cancer. Gynecol. Oncol. *167*, 334–341. https://doi.org/10.1016/j.ygyno.2022.09.004.

41. Ignatiadis, M., Saloustros, E., Joaquim, A., Casas, J.G., Kelly, C.M., Razis, E., Lambertini, M., Foukakis, T., Fehr, M., Menke-van der Houven van Oordt, C.W., et al. (2024). 338TiP EORTC-2129-BCG: Elacestrant for treating ER+/HER2- breast cancer patients with ctDNA relapse (TREAT ctDNA). Ann. Oncol. *35*, S355–S356. https://doi.org/10.1016/j.annonc.2024.08.277.

42. Pantel, K., Lindbjerg Andersen, C., Schuuring, E., Malats, N., Heitzer, E., Vat, L., Kiernan, E., Chin, E., Barrett, J.C., Kapp, J.R., et al. (2023). 237TiP GUIDE.MRD: A Consortium guiding multi-modal therapies against minimal residual disease (MRD) by liquid biopsy to assess implementation of circulating tumor DNA (ctDNA) in clinical practice to improve patient outcomes. Ann. Oncol. *34*, S276–S277. https://doi.org/10.1016/j.annonc.2023.09.2960.

43. Li, Y., Xu, J., Hu, X., Chen, Y., Liu, F., Chen, Y., Ma, X., Dong, Q., Sun, L., Mo, S., et al. (2024). Personalized circulating tumor DNA monitoring improves recurrence surveillance and management after curative resection of colorectal liver metastases: a prospective cohort study. Int. J. Surg. *110*, 2776–2787. https://doi.org/10.1097/JS9.0000000000001236.

44. Liu, G., Huang, S.H., Ailles, L., Rey-McIntyre, K., Melton, C.A., Shen, S.Y., Burgener, J.M., Brown, B., Zhang, J., Min, J., et al. (2025). Clinical validation of a tissue-agnostic genome-wide methylome enrichment molecular residual disease assay for head and neck malignancies. Ann. Oncol. *36*, 108–117. https://doi.org/10.1016/j.annonc.2024.08.2348.

45. Nakamura, Y., Kaneva, K., Lo, C., Neems, D., Freaney, J.E., Boulos, H., Hyun, S.W., Islam, F., Yamada-Hanff, J., Driessen, T.M., et al. (2025). A Tumor-Naive ctDNA Assay Detects Minimal Residual Disease in Resected Stage II or III Colorectal Cancer and Predicts Recurrence: Subset Analysis from the GALAXY Study in CIRCULATE-Japan. Clin. Cancer Res. *31*, 328–338. https://doi.org/10.1158/1078-0432.CCR-24-2396.

46. Wang, S., Xia, Z., You, J., Gu, X., Meng, F., Chen, P., Tang, W., Bao, H., Zhang, J., Wu, X., et al. (2023). Enhanced Detection of Landmark Minimal Residual Disease in Lung Cancer Using Cell-free DNA Fragmentomics. Cancer Res. Commun. *3*, 933–942. https://doi.org/10.1158/2767-9764.CRC-22-0363.

47. Cohen, S.A., Liu, M.C., and Aleshin, A. (2023). Practical recommendations for using ctDNA in clinical decision making. Nature *619*, 259–268. https://doi.org/10.1038/s41586-023-06225-y.

48. Chen, T., Chen, X., Zhang, S., Zhu, J., Tang, B., Wang, A., Dong, L., Zhang, Z., Yu, C., Sun, Y., et al. (2021). The Genome Sequence Archive Family: Toward Explosive Data Growth and Diverse Data Types. Genom. Proteom. Bioinform. *19*, 578–583. https://doi.org/10.1016/j.gpb.2021.08.001.

49. Members, C.-N.; Partners (2025). Database Resources of the National Genomics Data Center, China National Center for Bioinformation in 2025. Nucleic Acids Res. *53*, D30–D44. https://doi.org/10.1093/nar/gkae978.

50. Wang, K., Li, M., and Hakonarson, H. (2010). ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. *38*, e164. https://doi.org/10.1093/nar/gkq603.

51. Cingolani, P., Platts, A., Wang, L.L., Coon, M., Nguyen, T., Wang, L., Land, S.J., Lu, X., and Ruden, D.M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin) *6*, 80–92. https://doi.org/10.4161/fly.19695.

52. Rice, T.W., Ishwaran, H., Ferguson, M.K., Blackstone, E.H., and Goldstraw, P. (2017). Cancer of the Esophagus and Esophagogastric Junction: An Eighth Edition Staging Primer. J. Thorac. Oncol. *12*, 36–42. https://doi.org/10.1016/j.jtho.2016.10.016.

53. Zhang, X., Eyck, B.M., Yang, Y., Liu, J., Chao, Y.K., Hou, M.M., Hung, T.M., Pang, Q., Yu, Z.T., Jiang, H., et al. (2020). Accuracy of detecting residual disease after neoadjuvant chemoradiotherapy for esophageal squamous cell carcinoma (preSINO trial): a prospective multicenter diagnostic cohort study. BMC Cancer *20*, 194. https://doi.org/10.1186/s12885-020-6669-y.

54. Chirieac, L.R., Swisher, S.G., Ajani, J.A., Komaki, R.R., Correa, A.M., Morris, J.S., Roth, J.A., Rashid, A., Hamilton, S.R., and Wu, T.T. (2005). Post-therapy pathologic stage predicts survival in patients with esophageal carcinoma receiving preoperative chemoradiation. Cancer *103*, 1347–1355. https://doi.org/10.1002/cncr.20916.

55. Mao, X., Zhang, Z., Zheng, X., Xie, F., Duan, F., Jiang, L., Chuai, S., Han-Zhang, H., Han, B., and Sun, J. (2017). Capture-Based Targeted Ultradeep Sequencing in Paired Tissue and Plasma Samples Demonstrates Differential Subclonal ctDNA-Releasing Capability in Advanced Lung Cancer. J. Thorac. Oncol. *12*, 663–672. https://doi.org/10.1016/j.jtho.2016.11.2235.

56. Zhang, Y., Zeng, L., Zhang, X., Li, Y., Liu, L., Xu, Q., Yang, H., Jiang, W., Lizaso, A., Qiu, L., et al. (2021). Clinical and molecular feature-based nomogram model for predicting benefit from bevacizumab combined with first-generation EGFR-tyrosine kinase inhibitor (TKI) in EGFR-mutant advanced NSCLC. BMC Med. *19*, 245. https://doi.org/10.1186/s12916-021-02118-x.

57. Liang, N., Li, B., Jia, Z., Wang, C., Wu, P., Zheng, T., Wang, Y., Qiu, F., Wu, Y., Su, J., et al. (2021). Ultrasensitive detection of circulating tumour DNA via deep methylation sequencing aided by machine learning. Nat. Biomed. Eng. *5*, 586–599. https://doi.org/10.1038/s41551-021-00746-5.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Pre-treatment tumor biopsy samples of esophageal squamous cell carcinoma patient (preSINO study)	This study	https://clinicaltrials.gov/ct2/show/NCT03937362
Blood samples of esophageal squamous cell carcinoma patient (preSINO study)	This study	https://clinicaltrials.gov/ct2/show/NCT03937362
Critical commercial assays		
HS UMI library prep kit	Burning Rock	Cat#RS07P-12
MagPure FFPE DNA/RNA LQ kit	Magen	Cat#D6323-500R
MagPure Universal DNA Kit	Magen	Cat#MD5105-02
QIAamp Circulating Nucleic Acid kit	Qiagen	Cat#55114
Deposited data		
ctDNA sequencing data of 132 ESCC patients	This paper	GSA-human: HRA010802 (https://ngdc.cncb.ac.cn/gsa-human/browse/HRA010802)
Whole-exome sequencing data of tumor tissue from 132 ESCC patients	This paper	GSA-human: HRA010802 (https://ngdc.cncb.ac.cn/gsa-human/browse/HRA010802)
Software and algorithms		
ANNOVAR (20160201)	Wang et al.[50]	RRID: SCR_012821
SnpEff (v4.3)	Cingolani et al.[51]	RRID: SCR_005191
R (v4.3.1)	R Foundation for Statistical Computing	https://www.r-project.org/
SPSS (v29.0)	IBM	https://www.ibm.com/spss
GraphPad Prism (v10.2.0)	GraphPad Software	https://www.graphpad.com/
Code used for bioinformatics analysis of tumor-informed personalized ctDNA assay	Chen et al.[13]	https://github.com/bnr-cdx/prophet
Other		
Cell-Free DNA BCT tubes	Streck	Cat# 230244
Novaseq 6000 System	Illumina	Cat# 20012850
The reference human genome (GRCh37; hg19)	NCBI	GCF_000001405.13

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Study design and participants

This study (Figures 1 and S1) was an exploratory analysis of the preSINO (pre-Surgery If Needed for Esophageal cancer) trial (ClinicalTrials.gov registration: NCT03937362, details provided in the next section).[19] This trial is a prospective multicenter diagnostic trial to assess the accuracy of conventional clinical diagnostic modalities for detecting residual disease after nCRT in ESCC at three high-volume Asian centers: Shanghai Chest Hospital (Shanghai), Queen Mary Hospital (Hong Kong), and Chang Gung Memorial Hospital-Linkou (Taiwan).

The inclusion criteria for eligible patients were: (1) gender of male or female, aged 20–75 years; (2) performance status (Eastern Cooperative Oncology Group (ECOG) score of 0–2; (3) histologically confirmed squamous-cell carcinoma of thoracic esophagus; (4) resectable locally advanced stage (T1N + M0, cT2-4aN0/+M0) based on the TNM classification (8th American Joint Committee for Cancer staging system)[52] before the receipt of any treatment; (5) able to undergo curative surgical resection preceded by nCRT according to the CROSS regimen consisting of 5 weekly cycles of carboplatin and paclitaxel with 41.4 Gy radiotherapy in 23 sessions.[3] Exclusion criteria included: patients with a second primary tumor, unresectable or metastatic disease, a history of other cancers, or a previous history of cancer treatment. In this study, all 132 participants were East Asian (Han Chinese). Gender-related data was based on self-report and was consistent with each participant's sex assigned at birth. This study cohort comprised 21 females (15.9%) and 111 males (84.1%). No gender-stratified analyses were conducted, as the study was not powered to evaluate gender-based differences, which we acknowledge as a limitation.

Details of the preSINO trial have been described previously.[19,53] In brief, after the completion of nCRT, participants were scheduled to undergo one or two CRE based on conventional diagnostic modalities for residual disease assessment after nCRT. CRE included upper gastrointestinal endoscopy with bite-on-bite biopsies of primary lesion and EUS-FNA of suspected lymph nodes. The detailed process is as follows (Figure S2): Four to six weeks after completion of nCRT, patients underwent a first CRE (CRE-1) with endoscopic bite-on-bite biopsies. In cases with residual disease (as proven by biopsies with pathologically vital tumor cells), as well as in case of biopsies with high-grade dysplasia or endoscopic non-traversable stenosis, CRE-1 was considered positive and the patient was identified as incomplete responder. These patients underwent immediate surgery if no distant metastases were present on PET-CT. In patients without histological evidence of residual tumor (negative biopsies), patients received a second CRE (CRE-2) at 10–12 weeks after completion of nCRT, consisting of endoscopic bite-on-bite biopsy, EUS-FNA, and PET-CT. Immediately after CRE-2, all patients underwent surgery unless distant metastases were found during the PET-CT scan. Only patients that proceeded to surgery were included for analysis as surgical pathology is required as the gold standard for pathological response diagnosis. Pathological responses from the resection specimen were collected. The combined accuracy of both CRE-1 and CRE-2 for detecting residual disease before surgery was correlated to the pathological response in the resection specimen (gold standard) for calculation of the diagnostic accuracy (e.g., sensitivity and false negative rate).

Our hypothesis, derived from findings in our prior preSANO trial,[26] was that conventional CRE methods (endoscopic biopsies and EUS-FNA) may not sufficiently detect systemic residual disease. Accordingly, ctDNA-based molecular response evaluation (MoRE) was incorporated as an exploratory endpoint in the preSINO trial. To perform ctDNA analysis, serial blood samples were prospectively collected at multiple prespecified time points: at baseline prior to nCRT, post-nCRT simultaneously with conventional CRE (CRE-1 and CRE-2), post-surgery (3–4 weeks [F1] and 3-to-6 months [F2]), whenever possible. Baseline tumor tissues prior to nCRT were also collected for whole-exome sequencing (WES) to design personalized ctDNA panels. Overall, between August 2019 and January 2023, of the 242 eligible patients included for the primary endpoint analysis of the preSINO trial, a total of 132 patients with available qualified paired tumor tissue and blood samples for ctDNA analysis were defined as the biomarker evaluable set (BES) and were included in the present study (Figure S1). The remaining patients were excluded from analysis due to the unavailability of baseline specimens, with reasons shown in Figure S1.

The objective of this study (Figure 1) was to assess the accuracy and clinical feasibility of ctDNA-based MoRE in predicting residual disease after nCRT and disease recurrence after surgery. This study comprised three parts: (1) comparing the performance of three ctDNA assays, including a tumor-informed personalized panel, a tumor-naïve fixed panel, and a tumor-informed fixed panel; (2) assessing the diagnostic value of ctDNA-based MoRE at post-nCRT time point, either alone or in combination with conventional diagnostic methods, for detecting residual disease; (3) evaluating the predictive ability of ctDNA-based MoRE at post-surgery time point following nCRT for predicting disease recurrence and guiding adjuvant therapy selection.

This study was approved by the Ethics Committee of Shanghai Chest Hospital (approval number: IS22006) and was performed in accordance with the Declaration of Helsinki in 1964 and its current amendments. All participants provided written informed consent.

METHOD DETAILS

Study outcomes and objectives

The treatment response to nCRT was defined by histopathologic examination of the resection specimens by experienced pathologist. The entire resected tumor and all lymph nodes were evaluated, and pathological tumor regression was assessed by estimating the percentage of residual viable tumor in the macroscopically identifiable tumor bed using the Chirieac tumor regression grade (TRG) system[54]: no residual tumor cells (TRG1), 1–10% residual tumor cells (TRG2), 11–50% residual tumor cells (TRG3), and more than 50% residual tumor cells (TRG4). Residual disease was divided into two categories: (1) non-pathological complete response (non-pCR), defined as any ($\geq$1%) residual cancer cells; (2) non-major pathological response (non-MPR), defined as >10% residual cancer cells of the primary tumor (TRG3–4) and/or any residual cancer cells of the lymph nodes, regardless of the primary tumor response (including TRG1–2ypN+). To assess the performance of ctDNA alone or in combination with conventional CRE to predict residual disease, sensitivity, specificity, false-negative rate (FNR), negative predictive value (NPV) and positive predictive value (PPV) were calculated.

Disease-free survival (DFS) was defined as the time from the surgery to recurrence or death due to any cause. Overall survival (OS) was defined as the time from surgery to death. To assess the performance of ctDNA to predict postoperative recurrence, postoperative recurrence rate, DFS and OS were compared between ctDNA positive and ctDNA negative groups.

Postoperative adjuvant immunotherapy

The administration of adjuvant immunotherapy in the present preSINO study was influenced by the evolving clinical guidelines and regulatory approvals during the study period (2019–2023). Based on the results of CheckMate577 trial,[28] adjuvant immunotherapy (Nivolumab, a PD-1 inhibitor) was recommended for completely resected esophageal cancer with any pathologic residual disease (referred to non-pCR) in patients who had received nCRT by the NCCN Guidelines for the Esophageal and Esophagogastric Junction Cancers in 2022 and by the Chinese Society of Clinical Oncology Guidelines in 2022. In current clinical practice, patients with pCR generally do not receive adjuvant immunotherapy, while those with non-pCR are recommended to undergo it.[29]

In this study, patient enrollment spanned from August 2019 to January 2023; at the time of study initiation in 2019, there were no established adjuvant treatment recommendations for esophageal cancer patients following nCRT and surgery. Therefore, the study design did not mandate postoperative adjuvant therapy. As a result, none of the patients with pCR in our study received adjuvant immunotherapy, consistent with current clinical practice. For non-pCR patients enrolled between 2019 and 2021, adjuvant immunotherapy was not routinely administered, as it had not yet been endorsed by clinical guidelines or regulatory agencies. However, following the publication of CheckMate-577 trial and the subsequent updates to the NCCN and China guidelines, some non-pCR patients enrolled between 2022 and 2023 did receive adjuvant immunotherapy. This timeline reflects the dynamic nature of clinical practice and guideline updates during the study period. The differential administration of adjuvant immunotherapy in non-pCR patients is a result of the study's longitudinal design, which spanned a period of evolving evidence and regulatory approvals.

Postoperative follow-up

The first follow-up visit was 1 month after surgery. Patients were then regularly followed up every 3–6 months during the first 2-year period, and every 6–12 months thereafter. In the present study, the cutoff date for follow-up data collection was January 2025, with a minimum follow-up period of 20 months and a maximum of 48 months. Followed-up evaluations included physical examinations, routine blood tests, chest-abdominal CT scan, and ultrasonography of the neck. Upper gastrointestinal endoscopy was performed every 6 months in the first 2 years and once a year thereafter. Additional examinations were conducted based on the judgments of the attending physician.

Based on the first relapse pattern, recurrences were classified as locoregional or distant disease. Locoregional recurrences were defined as recurrences within the esophagus, anastomosis site, or regional lymph nodes. Distant recurrences were defined as hematogenous organ metastases (such as lung, liver, and bone), peritoneal carcinomatosis, or malignant pleural effusions.

Sample collection, processing and sequencing

All sample collection procedures complied with the regular routine in clinical practice and written informed consent was obtained before sampling.

Pretreatment tumor samples were obtained for WES from the primary esophageal tumor lesion before nCRT via endoscopic biopsy. Tumor tissue samples that contained less than 30% cancer cells were excluded (Figure S1). Tumor genomic DNA (gDNA) was extracted from formalin-fixed, paraffin-embedded (FFPE) tumor tissue specimens using MagPure FFPE DNA/RNA LQ kit (Magen, Guangzhou, China). Matched genomic DNA was extracted from EDTA-anticoagulated peripheral whole blood or buffy coat samples using MagPure Universal DNA Kit (Magen, Guangzhou, China) according to the manufacturer's instructions. Subsequently, 50–200 ng DNA samples underwent WES library preparation, exome capture, quantification and sequencing on a NovaSeq 6000 platform (Illumina, San Diego, CA, US), with 2 $\times$ 151bp paired-end reads and a mean target coverage of 500x for tumor samples and 150x for paired normal samples, as previously described.[13]

Approximately 20 mL of peripheral blood at each predefined time point was collected by Cell-Free DNA BCT tubes (Streck, La Vista, NE, USA). The collected blood sample was centrifuged at 2,000 g at 4°C for 10 min within 72h of collection. The supernatant plasma was then transferred to a 15mL centrifuge tube and centrifuged at 16,000 g at 4°C for 10 min. The supernatant was then transferred to a new tube and stored at −80°C for further analysis. Plasma circulating cell-free DNA (cfDNA) was extracted using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany). 20–60 ng cfDNA was subjected to three ctDNA assays of MRD. Ultra-deep UMI-based sequencing was performed on a NovaSeq 6000 platform (Illumina, San Diego, CA, US), with 2 $\times$ 151bp paired-end reads and a target raw depth of 100,000x for the personalized panel and 35,000x for the fixed panel.

Personalized ctDNA assay for MRD detection

The tumor-informed personalized panel used in this study was based on the CanCatch (PROPHET) platform (Burning Rock Biotech, Guangzhou, China), which has been fully described in our previous publication.[13] The complete bioinformatics algorithm analysis code for measuring ctDNA is publicly available at GitHub (https://github.com/bnr-cdx/prophet).

Patient-specific somatic variants were identified through the analysis of WES data obtained from the primary tumor and matched normal white blood cell. The analysis of WES data was as previously described.[13] In brief, after adapter trim, mapping, quality-control (QC) process, single nucleotide variants (SNVs) and insertions/deletions (INDELs) were called if the variant supporting reads$\geq$5 and mutation variant allele frequency (VAF)$\geq$3%. Variants with a population frequency>0.5% in tumors were excluded as single-nucleotide polymorphisms (SNPs). To exclude germline mutations, we calculated the fold change of tumor VAF and its paired-normal VAF. All variants with the fold change less than 3 or both VAFs greater than 10% were excluded. All variants passing the applied filters were annotated with ANNOVAR[50] and SnpEff v4.3.[51]

Up to 50 highly ranked variants with a VAF$\geq$3% were selected with in-house priority rules. Additionally, variants falling in the repetitive regions, regions with a high GC content (>75%), and homologous regions were filtered out. The biotinylated capture probe pool was produced in-house based on each personalized panel design.

The cfDNA was sequenced by the personalized panel and the sequencing data were extracted by UMI adapters, mapped, and filtered. The assumption of a Poisson distribution was used to calculate the significance of somatic mutations for the tumor-informed personalized panel, as previously described.[13] Significant sites were defined as those with p value < 0.05. The sample significance was determined by chi-square distribution. The ctDNA MRD positive status based on the personalized panel was defined as having

two or more significant sites and a sample-level p value < 0.005. The ctDNA fraction in plasma samples was estimated based on multiple loci using the maximum likelihood (ML) method, as previously described.[13]

Somatic mutation caller for fixed targeted panel

The fixed panel spanning 273 kb of the human genome, covering 168 genes (Burning Rock Biotech, Guangzhou, China; Table S3 provides the gene list)[55,56] that are frequently mutated in common cancers was used, which has been validated in the Food and Drug Administration (FDA)-led Sequencing Quality Control Phase 2 (SEQC2) project.[20] The raw sequencing data analysis and tumor-agnostic somatic mutation calling were performed with in-house bioinformatics pipeline, as previously described.[57]

For the tumor-naïve fixed panel, MRD was considered positive if any of the following criteria were met: (1) Driver SNV/INDEL≥1 high-quality reads (family size≥2 or duplex size≥1) and AF≥0.01%. (2) Passenger SNV/INDEL≥2 high-quality reads (family size≥2 or duplex size≥1) and AF≥0.1%. (3) Driver fusion≥3 high-quality reads or passenger fusion≥15 high-quality reads.

For the tumor-informed fixed panel, paired tumor tissue was used to guide variant calling. Specifically, in plasma samples, any SNV/INDEL not detected in the paired tumor tissue was considered a false positive and excluded from MRD reporting. Otherwise, SNV/INDELs with supporting reads≥4 or VAF ≥0.1% were considered positive and reported.

The analytical pipeline, including sequencing platform, panel design, bioinformatics filtering, and variant interpretation logic, was also reported in previously publication.[13]

QUANTIFICATION AND STATISTICAL ANALYSIS

Continuous variables were compared using the Mann-Whitney or Kruskal–Wallis test in cases of nonnormal distribution. Categorical variables were compared using the Chi-square test or Fisher's exact test, as appropriate. Survival was estimated by the Kaplan-Meier method, and differences of survival between groups were compared with the log rank test. Hazard ratios (HR) with corresponding 95% confidence intervals (CI) were estimated using the univariable Cox proportional-hazards regression model. Multivariable Cox proportional-hazards regression model was used to assess the association between variables and survival, adjusting for potential confounders. We used R (version 4.4.1), SPSS (version 29.0) and GraphPad Prism (version 10.2.0) for data analyses and graphical plotting. A p-value<0.05 was considered statistically significant. Details of statistical tests for the various analyses are described in the relevant methods section and in the respective figure legends.

ADDITIONAL RESOURCES

The study has been registered on Clinicaltrials.gov (NCT03937362): https://clinicaltrials.gov/study/NCT03937362.