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BIOMARKERS

Analysis of Circulating Tumor DNA to Predict Risk of Recurrence in Patients With Esophageal and Gastric Cancers

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original reports abstract

PURPOSE Circulating tumor DNA (ctDNA) analyses allow for postoperative risk stratification in patients with curatively treated colon and breast cancers. Use of ctDNA in esophagogastric cancers (EGC) is less characterized and could identify high-risk patients who have been treated with curative intent.

METHODS In this retrospective analysis of real-world data, ctDNA levels were analyzed in the preoperative, postoperative, and surveillance settings in patients with EGC using a personalized multiplex polymerase chain reaction–based next-generation sequencing assay. Plasma samples (n = 943) from 295 patients at > 70 institutions were collected before surgery, postoperatively, and/or serially during routine clinical follow-up from September 19, 2019, to February 21, 2022. ctDNA detection was annotated to clinicopathologic features and recurrence-free survival.

RESULTS A total of 295 patients with EGC were analyzed, and 212 patients with stages I-III disease were further explored. Pretreatment ctDNA was detected in 96% (23/24) of patients with preoperative time points. Postoperative ctDNA was detected in 23.5% (16/68) of patients with stage I-III EGC within 16 weeks (molecular residual disease window) after surgery without receiving systemic therapy. ctDNA detection at any time point after surgery (hazard ratio [HR], 23.6; 95% CI, 10.2 to 66.0; $P < .0001$), within the molecular residual disease window (HR, 10.7; 95% CI, 4.3 to 29.3; $P < .0001$), and during the surveillance period (HR, 17.7; 95% CI, 7.3 to 50.7; $P < .0001$) was associated with shorter recurrence-free survival. In multivariable analysis, ctDNA status and clinical stage of disease were independently associated with outcomes.

CONCLUSION Using real-world data, we demonstrate that postoperative tumor-informed ctDNA detection in EGC is feasible and allows for enhanced patient risk stratification and prognostication during curative-intent therapy.

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INTRODUCTION

Esophageal and gastric cancers (EGCs) are the sixth most common cancers worldwide.[1] In the United States, EGCs are expected to affect nearly 47,000 patients in 2022, leading to death in 27,500 patients.[2] In patients with localized disease who are treated with curative-intent therapy, over 50% recur within three years.[3-5] It is hypothesized that micrometastatic disease present at the time of surgical resection underlies the majority of recurrences, most of which are at distant sites.[6,7] Consequently, patients who have metastatic disease have a shorter overall survival of 12-14 months even with modern therapies.[8-11] There remains a significant unmet need to improve therapies for locoregional and advanced EGCs.

The curative-intent paradigm in EGC is associated with high patient morbidity, and optimal risk stratification is important for patient selection. Most patients initially present with large primary tumors or node-positive disease.[12] Even with current standards, curative-intent approaches yield a pathologic complete response (pCR) in only 23% of adenocarcinoma patients with EGC treated with neoadjuvant chemoradiation (CROSS regimen) and 16% with chemotherapy alone.[13,14] The majority of patients with esophageal cancer have residual disease after chemoradiation and have a median disease-free survival of only 11 months.[5,14] In patients receiving adjuvant immunotherapy with nivolumab, the median disease-free survival improves to 22.4 months.[5] Similarly < one in five patients with EGC treated with the perioperative fluorouracil plus leucovorin, oxaliplatin, and docetaxel (FLOT4) regimen exhibit a pCR, and conventional histopathologic and radiographic features are inadequate predictors of recurrence.[13] Given these factors, sensitive biomarkers are needed to better identify patients at higher risk for recurrence.

Circulating tumor DNA (ctDNA) has emerged as a noninvasive biomarker to assess recurrence risk in various malignancies.[15-18] The use of ctDNA in EGC to

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

Does circulating tumor DNA (ctDNA) quantification and analysis enable postoperative risk stratification and prediction of recurrence in patients with esophagogastric cancers?

Knowledge Generated

Among 943 plasma samples collected from 295 patients, the presence of ctDNA postoperatively was strongly associated with disease recurrence. Additionally, after adjusting for several known clinicopathologic risk factors, ctDNA was independently associated with recurrent disease.

Relevance

Longitudinal assessment of ctDNA allows for accurate postoperative risk stratification and adjuvant therapy monitoring in patients with esophagogastric cancers.

identify patients at risk for recurrence has been limited by small cohorts and varying assays and time points, although data published to date suggest feasibility.[19-24] Beyond the immediate postcurative-intent setting, ctDNA may also be useful for serial surveillance purposes. Hypothetically, earlier detection of recurrence can lead to earlier intervention, leading to improved outcomes. To substantially expand on studies of ctDNA in EGC, we sought to determine the performance of ctDNA in detecting molecular residual disease (MRD) postoperatively and its association with recurrence-free survival (RFS) using a tumor-informed assay.

METHODS

Study Population

In this retrospective analysis of real-world data in patients with EGC from > 70 institutions, plasma samples were collected before surgery, postoperatively (within an MRD window defined as samples obtained within 16 weeks from surgery and before systemic therapy), and serially during routine clinical follow-up from September 19, 2019, to February 21, 2022. Tumor tissue was collected at resection or at initial diagnosis. Blood samples were collected longitudinally at the discretion of the clinician during routine clinical care. Clinicopathologic information was collected for all patients. All patients received treatment and follow-up in accordance with standard clinical practice and per the investigator's discretion. The complete clinical course of the cohort is depicted in Appendix Figure A1. Informed consent was obtained as part of the ordering assay. This study was approved by the corresponding Ethical and Independent Review Services (protocol# 20-049-ALL) and was conducted in accordance with the Declaration of Helsinki.

Personalized Multiplex-Polymerase Chain Reaction–Based Next-Generation Sequencing Assay for ctDNA Detection

A personalized, tumor-informed, multiplex (m)-polymerase chain reaction (PCR) next-generation sequencing (NGS) assay (Signatera) was used for the detection of ctDNA, as previously published.[15] Briefly, whole-exome sequencing was performed on formalin-fixed paraffin-embedded tumor blocks and matched-normal DNA blood samples. On the basis of the results of whole-exome sequencing, 16 patient-specific, somatic single-nucleotide variants were selected for each patient, and PCR primers were designed. Cell-free DNA was extracted from a median of 10 mL of plasma (range, 0.7-10.2 mL). Universal libraries were created by end repair, A-tailing, and ligation with custom adapters. Next, libraries were amplified by multiplex PCR, barcoded, pooled, and sequenced on a NGS platform. Samples with at least two tumor-specific variants were defined as ctDNA-positive, and ctDNA concentration was reported in mean tumor molecules/mL of plasma.

Statistical Analysis

Consistent with the International Society for Pharmacoeconomics and Outcomes Research guidelines, the inclusion and exclusion criteria, potential biases, primary and exploratory outcome measures, handling of missing data, etc were determined before analysis unless otherwise specified. The ctDNA statistical analysis plan was developed before unblinding the clinical data. Data were deidentified before analysis. The primary outcome was RFS, measured from the date of surgery to the first documented sign of radiologic recurrence, either locoregional or distant, or death from all causes, and was censored at last follow-up. Patients with < 10 days of clinical follow-up were excluded. Survival analysis was performed using Firth's penalized maximum likelihood bias reduction method for Cox regression in R (version 4.1) package *coxphf*.[25] A multivariable Cox proportional hazards model was used to explore the effects of clinicopathologic factors on RFS. All *P* values were based on two-sided testing; differences were considered significant at $P \leq .05$.

RESULTS

Patient Cohort

A total of 943 plasma samples (n) were collected from 295 patients (N) with esophageal (N = 86 patients, n = 288 samples), gastroesophageal junction (GEJ, N = 85,

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n = 279), and gastric (N = 124, n = 376) cancers. Since stage IV patients (N = 83, n = 249) rarely undergo curative-intent surgery, they were excluded from the survival analysis as shown in Figure 1. Of the remaining 212 patients with stage I-III EGC, cohorts were divided into three subgroups for survival analysis, on the basis of defined criteria: (1) MRD window (N = 68), defined as time points available within 16 weeks of surgery, before systemic therapy, (2) anytime ctDNA-positivity (N = 125), defined as ctDNA-positivity at any time after surgery regardless of treatment, and (3) surveillance (N = 84), for patients who received systemic therapy, with time points available after the end of 2 weeks of systemic therapy (Fig 1). Most patients had longitudinal time points available with ctDNA levels correlating to response to disease-directed treatment, ie, radiation, surgery, and/or systemic therapy, and were considered for survival analysis specific to their subgroup (MRD window, anytime ctDNA-positivity, and surveillance; Fig 1).

The MRD window of 16 weeks was chosen to reflect the time from curative-intent surgery to when clinicians need to decide on adjuvant therapy and is consistent with the window used in adjuvant clinical trials.[5] Cohort demographics and ctDNA analysis performed in each setting are described in Appendix Table A1.

ctDNA Detection Rates at Preoperative and Postoperative Time Points

Benchmarking ctDNA detection rates before and after therapy is central to informing the feasibility of novel ctDNA-guided neoadjuvant approaches. Among 212 patients with localized EGC (stage I-III), we identified 65 patients with esophageal (n = 234 plasma samples), 59 patients with GEJ (n = 188 plasma samples), and 88 patients with gastric cancer (n = 271 plasma samples). At diagnosis (baseline before treatment), ctDNA was detected in 96% (23/24) of patients. Table 1 presents ctDNA detection by anatomic location, histology, and disease stage.

Postoperative ctDNA Presence Is Associated With Increased Risk of Recurrence

In patients (N = 125; 36 esophageal, 32 GEJ, and 57 gastric) analyzed at any time point postoperatively (regardless of adjuvant treatment), the recurrence rate was 88.2% (30/34) in ctDNA-positive patients compared with 5.5% (5/91) in ctDNA-negative patients, exhibiting a marked reduction in RFS (median RFS 9.6 months for ctDNA-positive patients, median not reached in ctDNA-negative cohort; hazard ratio [HR], 23.6; 95% CI, 10.2 to 66.0; $P < .0001$; median follow-up time 12.2 months; Fig 2A). This trend was observed across all subtypes (Figs 2B-2D). Here, the ctDNA assay identified recurrence with a sensitivity of 85.7% (30/35) and a specificity of 95.5% (85/89). In multivariable analysis, ctDNA-positivity (HR, 11.82; 95% CI, 6.18 to 22.6; $P < .001$) and clinical stage III disease (HR, 2.82; 95% CI, 1.52 to 5.2; $P < .001$) were independently associated with worse RFS (Fig 2E).

Because anytime ctDNA-positivity encompasses a longer follow-up period, we sought to further understand evidence of residual disease by restricting analysis to patients in the postoperative MRD window, ie, within 16 weeks of surgery before adjuvant treatment (N = 68; 22 esophageal, 20 GEJ, and 26 gastric). ctDNA was detectable in 23.5% of patients tested (16/68) and in 24.6% (31/126) of all samples tested, suggesting that nearly one in four patients were ctDNA-positive after curative-intent surgery. The presence of ctDNA was associated with a higher recurrence rate of 81.2% (13/16) in comparison with a recurrence rate of 13.5% (7/52) in ctDNA-negative patients. Furthermore,

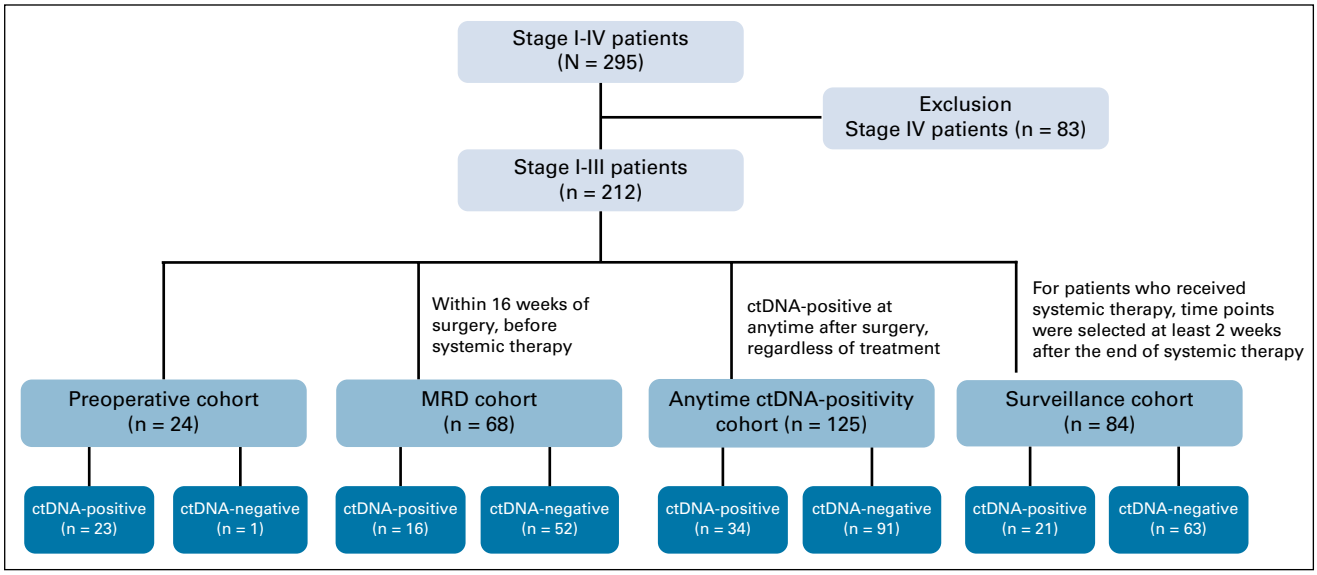

FIG 1. Flow diagram depicting an overview of number of patients and plasma samples included in the survival analysis. ctDNA, circulating tumor DNA; MRD, molecular residual disease.

TABLE 1. ctDNA Detection Rates and Quantification at Sample Level

Location	Histology	Stage	Baseline (n = 25), n/N (%)	MRD Window (n = 124), n/N (%)	On Treatment (n = 358), n/N (%)	Surveillance (n = 430), n/N (%)	Anytime Postoperative ctDNA-Positivity, n/N (%)
Esophageal (patients = 86, plasma = 288)	Adenocarcinoma (patients = 66, plasma = 236)	I (patients = 9, plasma = 30)	NA	1/7 (14.3)	2/3 (66.7)	1/20 (5)	4/30 (13.3)
		II (patients = 20, plasma = 91)	2/2 (100)	2/10 (20)	21/39 (53.8)	4/40 (10)	29/91 (31.9)
		III (patients = 22, plasma = 75)	1/1 (100)	5/8 (62.5)	23/33 (69.7)	6/33 (18.2)	35/75 (46.7)
	Small cell (patients = 1, plasma = 4)	III (patients = 1, plasma = 4)	NA	0/1 (0)	NA	0/3 (0)	0/4 (0.0)
	Squamous (patients = 19, plasma = 48)	II (patients = 3, plasma = 5)	1/1 (100)	0/1 (0)	NA	1/3 (33.3)	2/5 (40.0)
		III (patients = 10, plasma = 29)	1/1 (100)	0/6 (0)	2/7 (28.6)	1/15 (6.7)	4/29 (13.8)
GEJ (patients = 85, plasma = 279)	Adenocarcinoma (patients = 84, plasma = 278)	I (patients = 5, plasma = 19)	NA	2/3 (66.7)	8/8 (100)	0/8 (0)	10/19 (52.6)
		II (patients = 12, plasma = 35)	NA	3/9 (33.3)	4/7 (57.1)	2/19 (10.5)	9/35 (25.7)
		III (patients = 42, plasma = 134)	3/3 (100)	3/22 (13.6)	13/33 (39.4)	13/76 (17.1)	32/134 (23.9)
Gastric (patients = 124, plasma = 376)	Adenocarcinoma (patients = 123, plasma = 370)	I (patients = 15, plasma = 48)	NA	0/7 (0)	2/3 (66.7)	5/38 (13.2)	7/48 (14.6)
		II (patients = 28, plasma = 86)	1/2 (50)	1/14 (7.1)	3/8 (37.5)	9/62 (14.5)	14/86 (16.3)
		III (patients = 44, plasma = 131)	1/1 (100)	4/19 (21.1)	12/35 (34.3)	13/76 (17.1)	30/131 (22.9)
	Squamous (patients = 1, plasma = 6)	II (patients = 1, plasma = 6)	NA	NA	NA	0/6 (0)	0/6 (0.0)

Abbreviations: ctDNA, circulating tumor DNA; GEJ, gastroesophageal junction; MRD, molecular residual disease.

ctDNA-positive patients exhibited an inferior RFS (median RFS 6.0 months for ctDNA-positive, median not reached for ctDNA-negative; HR, 10.7; 95% CI, 4.3 to 29.3; $P < .0001$; median follow-up time 8.3 months; Fig 3A). This trend was observed across all anatomic subtypes (Figs 3B-3D), suggesting shared prognostic ability across biologically heterogeneous tumors. Of note, some of the ctDNA-positive patients were subsequently treated and became ctDNA-negative and did not relapse (Appendix Fig A1) but this should be considered a pilot observation, given treatment and follow-up heterogeneity.

In the surveillance setting (> 2 weeks after the completion of adjuvant treatment), the recurrence rate in patients (N = 84; 21 esophageal, 23 GEJ, and 40 gastric) with ctDNA-positivity was 95.2% (20/21) compared with 7.9% (5/63) in ctDNA-negative patients and demonstrated an inferior RFS (median RFS 10.8 months for ctDNA-positive *v* median not reached for ctDNA-negative; HR, 17.7; 95% CI, 7.3 to 50.7; $P < .0001$; median follow-up time 15.7 months; Fig 4A). This trend was observed across all subtypes (Figs 4B-4D). In this setting, the assay detected recurrence with a sensitivity of 80% (20/25) and a specificity of 98.3% (58/59).

Patient Case Study

To provide a patient-level example of how ctDNA may complement and ultimately improve upon current clinical management standards, we offer the following case example. Briefly, in 2019, a 56-year-old man presented with clinical cT3N1M0 (stage III) esophageal adenocarcinoma and was treated with standard neoadjuvant chemoradiation followed by R0 esophagectomy revealing a ypT1aypN1 residual adenocarcinoma with significant treatment effect (TRG 1) and 2/39 LN+ for disease. The patient recovered uneventfully and was started on standard-of-care radiographic and clinical surveillance. Outside of this treatment, the patient underwent serial ctDNA collection (Fig 5). Serial surveillance computed tomography (CT) imaging was notable for fluctuating

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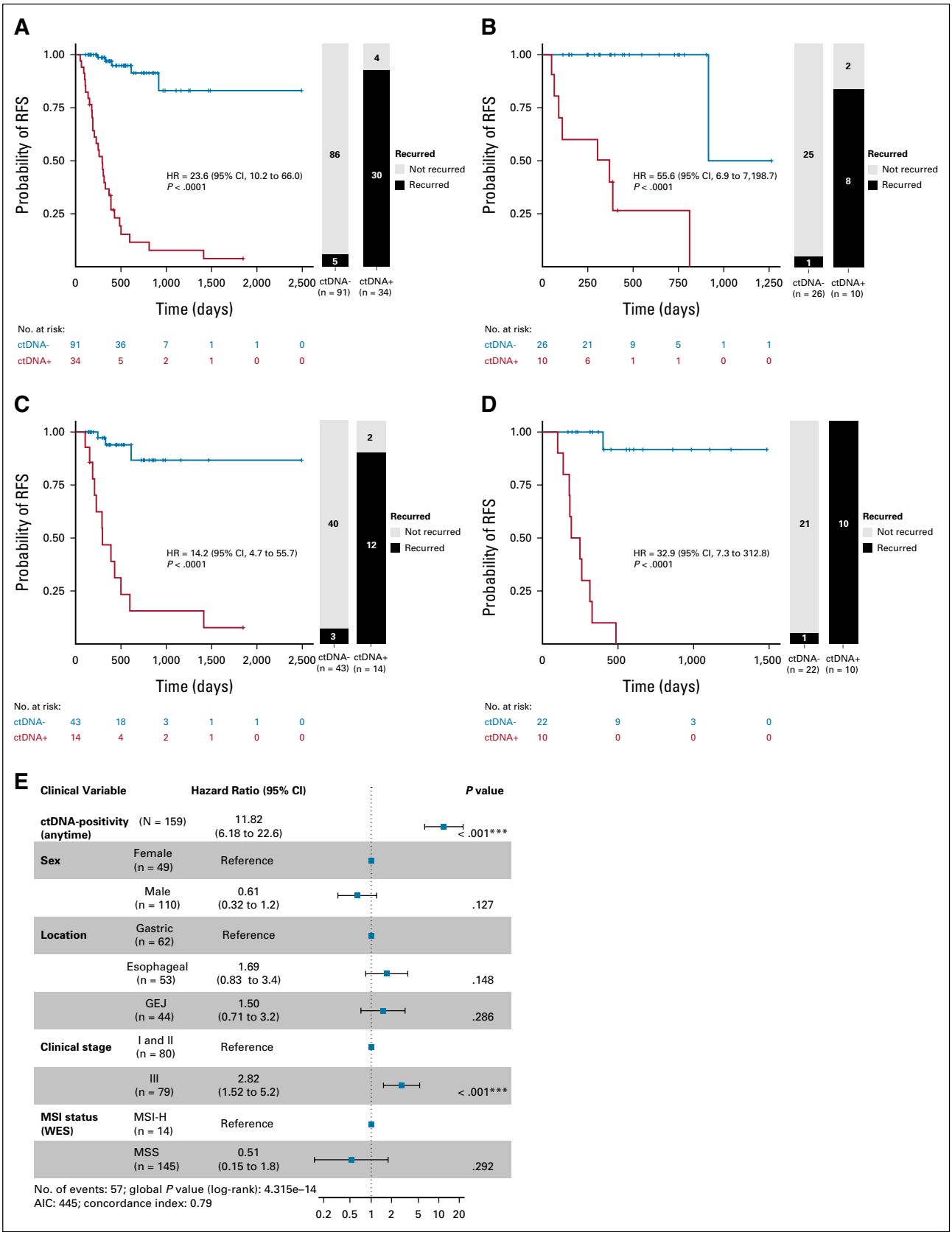

FIG 2. Kaplan-Meier estimates of patients with esophagogastric cancers representing RFS as stratified by anytime ctDNA-positivity and association of ctDNA with various prognostic factors and RFS: (A) all subtypes, (B) esophageal, (C) gastric, and (D) GEJ. ctDNA-positivity at any time postoperatively was significantly associated with poorer RFS. (E) Multivariate analysis of prognostic factors (continued on following page)

FIG 2. (Continued). and their association with RFS, as indicated by HR, analyzed across the cohort. AIC, akaike information criterion; ctDNA, circulating tumor DNA; GEJ, gastroesophageal junction; HR, hazard ratio; MSI, microsatellite instability; MSI-H, microsatellite instability high; MSS, microsatellite stable; RFS, recurrence-free survival.

subcentimeter pulmonary nodules radiographically perceived to represent microaspiration and/or inflammatory nodules, which are common in the postoperative setting.

Interestingly, ctDNA test performed approximately 5 months after surgery revealed a positive test despite negative contemporaneous CT scans. A repeat ctDNA test at a short interval remained positive with a rising mean tumor molecule value. Given the performance of similar assays in colorectal, bladder, and breast cancers, this result significantly raised the clinical suspicion for recurrence and prompted earlier repeat imaging than would otherwise have been performed per standard of care. Ultimately, the patient underwent a right upper lobe lung biopsy of a 9-mm nodule, which confirmed a metastatic recurrence. There were no other obvious sites of disease. This was followed with infusional fluorouracil, leucovorin, and oxaliplatin + nivolumab and stereotactic body radiotherapy (SBRT) to the biopsy-proven lung metastasis. Following SBRT, ctDNA became undetectable and the patient was transitioned to single-agent nivolumab maintenance. He

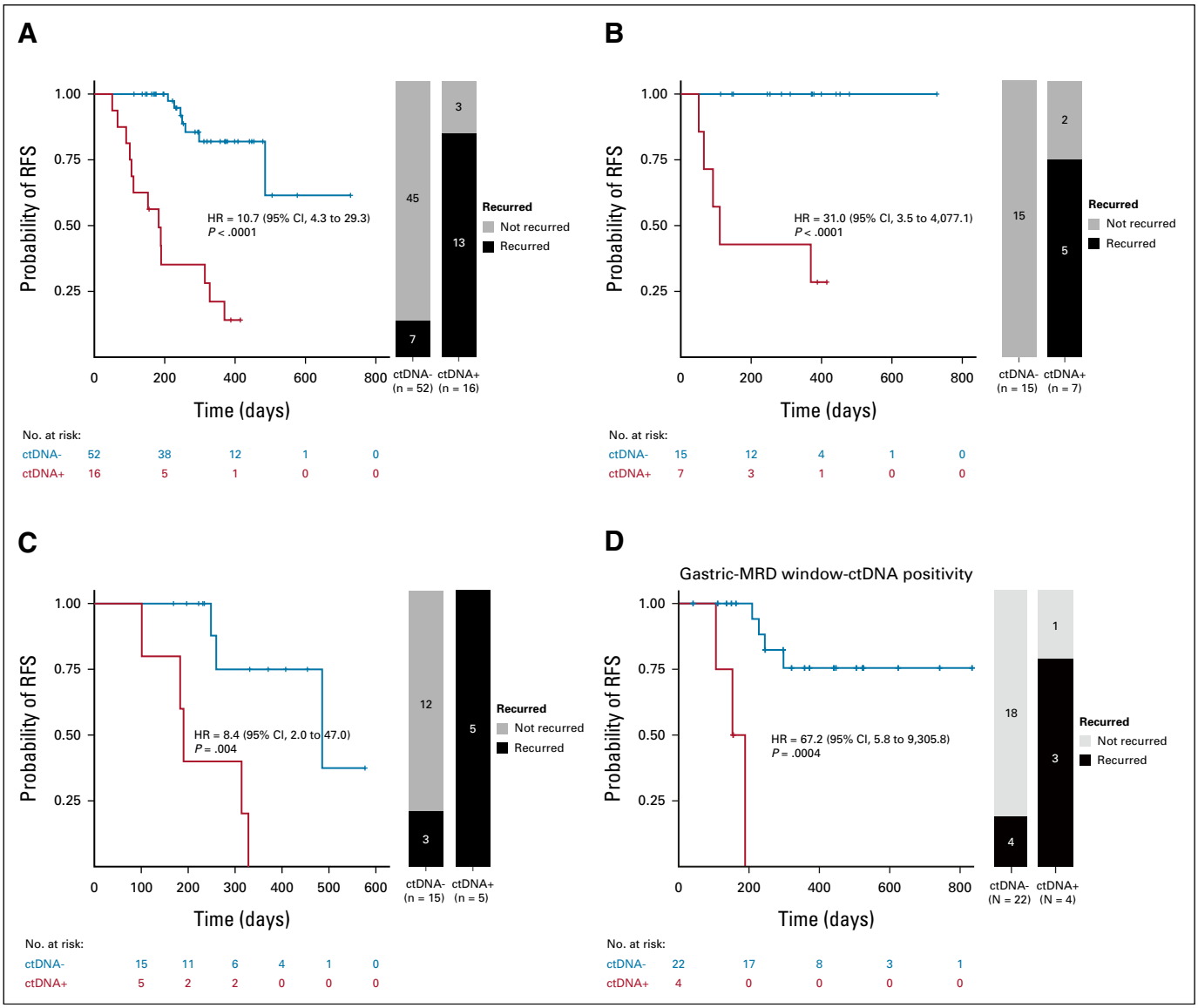

FIG 3. Kaplan-Meier estimates of patients with esophagogastric cancers representing RFS as stratified by ctDNA-positivity in the MRD window: (A) all subtypes, (B) esophageal, (C) gastroesophageal junction, and (D) gastric. ctDNA-positivity in the MRD window (within 16 weeks following surgery) was significantly associated with poorer RFS. ctDNA, circulating tumor DNA; HR, hazard ratio; MRD, molecular residual disease; RFS, recurrence-free survival.

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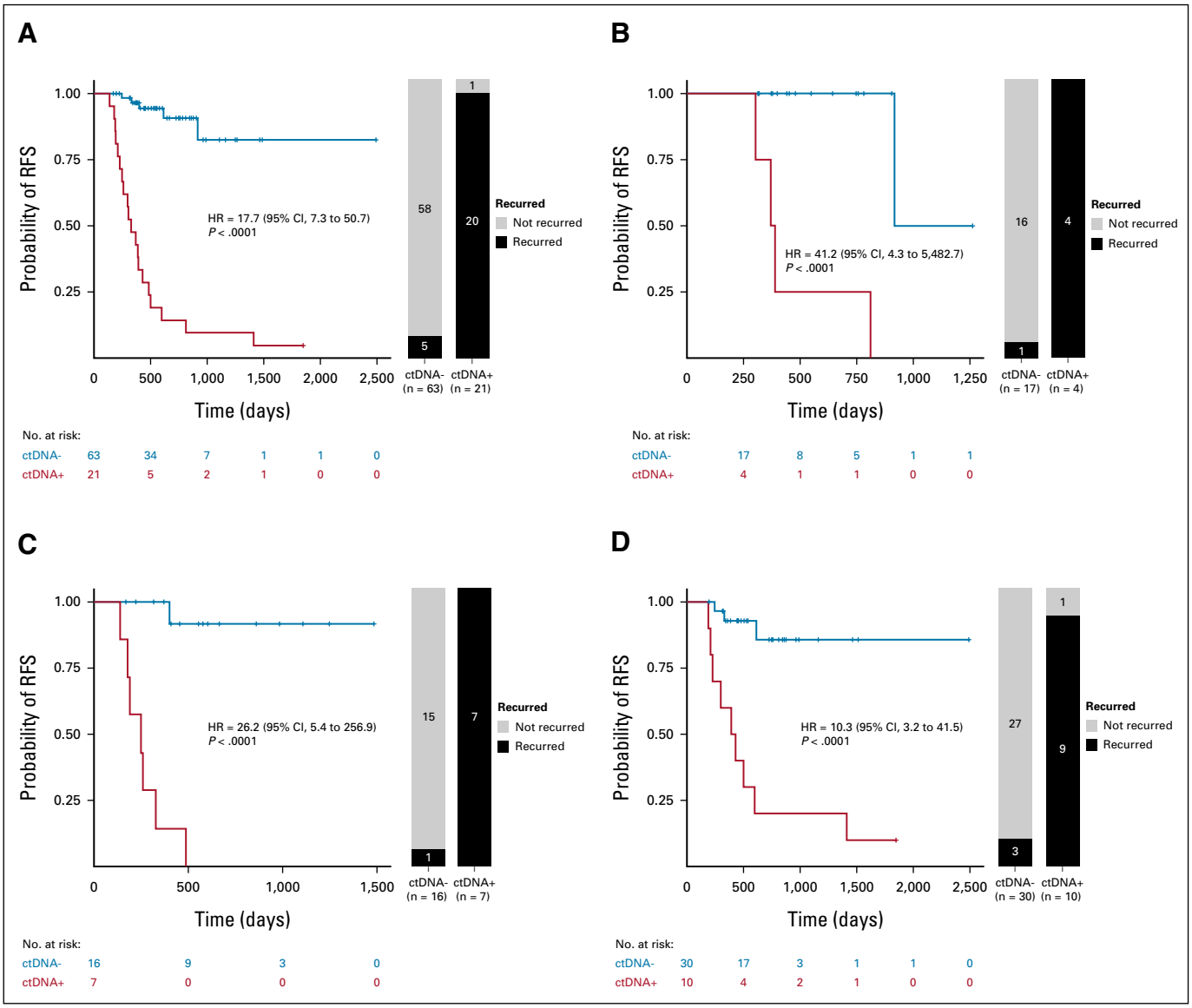

FIG 4. Kaplan-Meier estimates of patients with esophagogastric cancers representing RFS as stratified by ctDNA-positivity during the surveillance period: (A) all subtypes, (B) esophageal, (C) GEJ, and (D) gastric. ctDNA-positivity during the surveillance period was also significantly associated with decreased RFS. ctDNA, circulating tumor DNA; RFS, recurrence-free survival.

remained without radiographic disease and ctDNA-negative for 12 months, at which time his ctDNA became detectable again, prompting clinical imaging. CT showed no clear disease but was notable for a left lower-lobe nodule measuring 9 mm. After multidisciplinary discussions with medical oncology, radiation oncology, and thoracic surgery, the new lung lesion was determined to be the most likely site of disease and the patient underwent SBRT in approximately 28 months after surgery. At the most recent follow-up (approximately 30 months after surgery), ctDNA was undetectable (Fig 5). The patient was doing well with no radiographic evidence of disease maintained solely on nivolumab therapy.

Although this case should be considered highly preliminary, it does emphasize the potential clinical utility of ctDNA in EGC. Here, ctDNA helped guide surveillance and adjudicate the etiology of a subcentimeter lung nodule, which is otherwise common after esophagectomy. Additionally, this patient may highlight an oligometastatic paradigm in which serial ctDNA after ablative approaches can extend periods off chemotherapy further optimizing the quality of life for patients.

DISCUSSION

We investigated the performance of a personalized, tumor-informed ctDNA assay in a large, real-world cohort to risk-stratify curatively treated EGC patients. In our study, with a long follow-up and a large cohort, we demonstrate that detecting ctDNA at any time point postoperatively using a personalized, tumor-informed ctDNA assay is highly prognostic of poor outcomes, and identifies recurrence with high sensitivity (85.7%) and specificity (95.5%). Although some

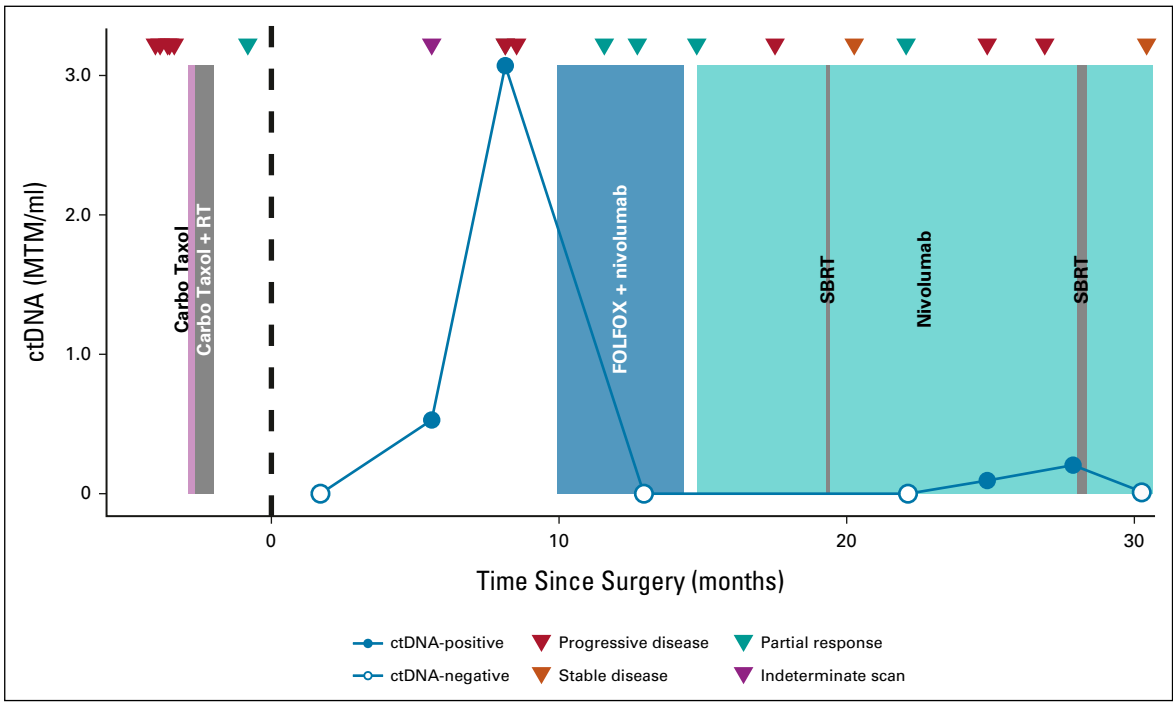

FIG 5. Case example: Postsurgical ctDNA dynamics along with radiologic findings were used to inform clinical decision making. ctDNA, circulating tumor DNA; FOLFOX, infusional fluorouracil, leucovorin, and oxaliplatin; MTM, mean tumor molecules; RT, radiation therapy; SBRT, stereotactic body radiation therapy.

studies have shown ctDNA-positivity to be associated with inferior patient outcomes, most of these studies have used a static gene panel–based NGS approach or droplet digital PCR, which may have lower sensitivity.[21,23,24,26-28]

Unlike predesigned ctDNA static panels, Signatera is a personalized, tumor-informed assay that relies on the prior knowledge of the mutational status of the patient's tumor. This tumor-informed approach ensures that MRD can be detected with both high sensitivity and specificity, reliably detecting variants down to 0.01% variant allele frequency.[15,29] This method also significantly reduces the false-positive rates by filtering out clonal hematopoiesis of indeterminate potential (CHIP) and germline-derived variants. Specifically, Ococks et al[22] found that of 97 patients with esophageal adenocarcinoma treated with curative intent, 21% of patients were ctDNA-positive following resection, and 75% of those patients developed recurrence. However, the authors concluded that the presence of CHIP variants may have confounded the results since the assay was not tumor-informed, although 65% of patients had peripheral blood available to exclude CHIP variants. In another study that involved 50 patients with gastric cancer from the CRITICS trial, a subcohort analysis (N = 20) showed a significant improvement in the association between cell-free DNA-positivity and overall survival after filtering out CHIP variants, ie, from HR 3.3 (95% CI, 0.4 to 29; $P = .28$) to HR 21.8 (95% CI, 3.9 to 123.1; $P = .001$).[20] Our data add to these recently published studies supporting the feasibility of ctDNA analysis in EGCs, with a median follow-up of 417 days (range, 7-2,491 days) and a larger number of patients.

Currently, decision making for adjuvant therapy after curative resection is determined by pathologic response and/or the ability to tolerate adjuvant therapy.[5,30] In our study, for patients with EGC, we defined the MRD window as samples drawn within 16 weeks of surgery. Since significant time is required for recovery after curative-intent surgery in patients with EGC, this window reflects the time period for clinical decisions around adjuvant therapy and was also used in the phase III CheckMate-577 EGC trial.[5,13] To maximize contextualization of our data, we paralleled this time frame to define the MRD window. We observed a significant proportion of patients in our study to be ctDNA-positive after surgery (23.5%), of whom 81.2% experienced recurrence. Reassuringly, our postsurgical ctDNA-positivity rate closely parallels the reported literature, suggesting there is a sizable portion of patients who could be considered for ctDNA-adapted adjuvant strategies.[22,31]

Notably, CheckMate-577 included only patients with incomplete pathologic response after neoadjuvant chemoradiation, given the known higher risk of recurrence in this subgroup. However, outcomes among pCR patients are also heterogeneous and warrant further study as we did observe recurrence among pCR patients. Because the clinical behavior and prognosis differs between squamous cell carcinoma, esophageal adenocarcinoma, and GEJ/gastric adenocarcinomas, we were intentional in analyzing our cohort collectively and also stratified by anatomic subgroups. Across anatomic sites, we

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observed an overall consistent ability of ctDNA to predict increased risk of recurrence, a finding not previously reported. Our data suggest that the use of ctDNA-positivity in addition to other clinicopathologic features for inclusion in the design of future EGC clinical trials is a feasible and attractive strategy.[32]

Although our analysis is focused on locoregional disease, we recognize ctDNA to have multiple potential applications across the treatment spectrum of EGC. We provide a pilot example to demonstrate the postcurative-intent application of ctDNA in surveillance and its use in complementing imaging in oligometastatic disease. This case parallels early data showing the ability of ctDNA to stratify patients with advanced colorectal cancer who undergo surgery.[16] We excluded stage IV patients from our primary analyses to avoid confounding the results but acknowledge potential future applications for ctDNA in riskstratifying and/or assessing response in oligometastatic approaches like the ongoing EA2183 phase III trial (ClinicalTrials.gov identifier: NCT04248452).[33]

Because of the real-world nature of this study, it has several limitations including patient and plasma collection heterogeneity and possible inherent selection bias. Several patients had shorter follow-up periods. However, with a median follow-up of 417 days (range, 7-2,491 days), our study has successfully addressed the main objective of observing the impact of MRD testing on adjuvant treatment decisions after surgery and assessing clinical outcomes. Furthermore, given the recurrence patterns in EGCs, we believe that the median follow-up presented in this study is within the clinically relevant time frame. Another limitation of the study was the inability to estimate the lead time from ctDNA-positivity to radiographic/clinical recurrence. Because of the pragmatic nature our study and availability of ctDNA results to treating clinicians, some clinicians may have altered their scanning frequency on the basis of the ctDNA results, thereby confounding the lead time. This would impact an accurate estimation of lead time in this data set. In a small retrospective study in patients with esophageal adenocarcinoma with the tumor-informed bespoke ctDNA assay, a median lead time of approximately 1 year was reported.[23] Additionally, we did observe a small number of patients who were ctDNA-negative who ultimately recurred, a phenomenon which has been observed in all MRD cohorts and likely reflects yet undefined biologic features and low ctDNA shedding.[34]

In summary, we highlight the prognostic role of ctDNA in patients with nonmetastatic EGC and help benchmark the ctDNA detection frequency in this population. These data represent the largest reported EGC cohort and could help refine the development of prospective studies required to validate our findings. Similar to its role in locoregional colon cancer, we envision a future in which ctDNA will improve outcomes in the neoadjuvant, adjuvant, surveillance, and advanced settings of EGC.

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PRIOR PRESENTATION

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SUPPORT

The analysis of commercial data was performed by Natera, Inc.

DATA SHARING STATEMENT

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

Conception and design: Brandon M. Huffman, Griffin L. Budde, Shifra Krinshpun, Adham Jurdi, Alexey Aleshin, Pashtoon M. Kasi, Samuel J. Klempner
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REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71:209-249, 2021
2. Siegel RL, Miller KD, Fuchs HE, Jemal A: Cancer statistics, 2022. CA Cancer J Clin 72:7-33, 2022
3. de Steur WO, van Amelsfoort RM, Hartgrink HH, et al: Adjuvant chemotherapy is superior to chemoradiation after D2 surgery for gastric cancer in the per-protocol analysis of the randomized CRITICS trial. Ann Oncol 32:360-367, 2021
4. Eyck BM, van Lanschot JJB, Hulshof MCCM, et al: Ten-year outcome of neoadjuvant chemoradiotherapy plus surgery for esophageal cancer: The randomized controlled CROSS trial. J Clin Oncol 39:1995-2004, 2021
5. Kelly RJ, Ajani JA, Kuzdzal J, et al: Adjuvant nivolumab in resected esophageal or gastroesophageal junction cancer. N Engl J Med 384:1191-1203, 2021
6. Jiao X, Krasna MJ: Clinical significance of micrometastasis in lung and esophageal cancer: A new paradigm in thoracic oncology. Ann Thorac Surg 74:278-284, 2002
7. Prenzel KL, Holscher AH, Drebber U, et al: Prognostic impact of nodal micrometastasis in early esophageal cancer. Eur J Surg Oncol 38:314-318, 2012
8. Bang Y-J, Van Cutsem E, Feyereislova A, et al: Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): A phase 3, open-label, randomised controlled trial. Lancet 376:687-697, 2010
9. Cunningham D, Starling N, Rao S, et al: Capecitabine and oxaliplatin for advanced esophagogastric cancer. N Engl J Med 358:36-46, 2008
10. Janjigian YY, Shitara K, Moehler M, et al: First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): A randomised, open-label, phase 3 trial. Lancet 398:27-40, 2021
11. Shitara K, Van Cutsem E, Bang YJ, et al: Efficacy and safety of pembrolizumab or pembrolizumab plus chemotherapy vs chemotherapy alone for patients with first-line, advanced gastric cancer: The KEYNOTE-062 phase 3 randomized clinical trial. JAMA Oncol 6:1571-1580, 2020
12. Then EO, Lopez M, Saleem S, et al: Esophageal cancer: An updated surveillance epidemiology and end results database analysis. World J Oncol 11:55-64, 2020
13. Al-Batran S-E, Hofheinz RD, Pauligk C, et al: Histopathological regression after neoadjuvant docetaxel, oxaliplatin, fluorouracil, and leucovorin versus epirubicin, cisplatin, and fluorouracil or capecitabine in patients with resectable gastric or gastro-oesophageal junction adenocarcinoma (FLOT4-AIO): Results from the phase 2 part of a multicentre, open-label, randomised phase 2/3 trial. Lancet Oncol 17:1697-1708, 2016
14. van Hagen P, Hulshof MC, van Lanschot JJ, et al: Preoperative chemoradiotherapy for esophageal or junctional cancer. N Engl J Med 366:2074-2084, 2012
15. Reinert T, Henriksen TV, Christensen E, et al: Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer. JAMA Oncol 5:1124-1131, 2019
16. Loupakis F, Sharma S, Derouazi M, et al: Detection of molecular residual disease using personalized circulating tumor DNA assay in patients with colorectal cancer undergoing resection of metastases. JCO Precis Oncol 5:1166-1177, 2021
17. Powles T, Assaf ZJ, Davarpanah N, et al: ctDNA guiding adjuvant immunotherapy in urothelial carcinoma. Nature 595:432-437, 2021
18. Magbanua MJM, Swigart LB, Wu HT, et al: Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. Ann Oncol 32:229-239, 2021
19. Einstein DJ, Liang N, Malhotra M, et al: Assessment of molecular remission in oligometastatic esophageal cancer with a personalized circulating tumor DNA assay. JCO Precis Oncol 4:239-243, 2020
20. Leal A, van Grieken NCT, Palsgrove DN, et al: White blood cell and cell-free DNA analyses for detection of residual disease in gastric cancer. Nat Commun 11:525, 2020
21. Maron SB, Chase LM, Lomnicki S, et al: Circulating tumor DNA sequencing analysis of gastroesophageal adenocarcinoma. Clin Cancer Res 25:7098-7112, 2019
22. Ococks E, Frankell AM, Masque Soler N, et al: Longitudinal tracking of 97 esophageal adenocarcinomas using liquid biopsy sampling. Ann Oncol 32:522-532, 2021
23. Ococks E, Sharma S, Ng AWT, et al: Serial circulating tumor DNA detection using a personalized, tumor-informed assay in esophageal adenocarcinoma patients following resection. Gastroenterology 161:1705-1708.e2, 2021
24. Wo JY, Clark JW, Eyler CE, et al: Results and molecular correlates from a pilot study of neoadjuvant induction FOLFIRINOX followed by chemoradiation and surgery for gastroesophageal adenocarcinomas. Clin Cancer Res 27:6343-6353, 2021
25. Heinze G, Ploner M, Jiricka L: coxphf: Cox Regression with Firth's Penalized Likelihood. R Package Version 1.13.1, 2020. https://cran.r-project.org/web/packages/coxphf/coxphf.pdf
26. Egyud M, Tejani M, Pennathur A, et al: Detection of circulating tumor DNA in plasma: A potential biomarker for esophageal adenocarcinoma. Ann Thorac Surg 108:343-349, 2019
27. Kato S, Okamura R, Baumgartner JM, et al: Analysis of circulating tumor DNA and clinical correlates in patients with esophageal, gastroesophageal junction, and gastric adenocarcinoma. Clin Cancer Res 24:6248-6256, 2018
28. Cabel L, Decraene C, Bieche I, et al: Limited sensitivity of circulating tumor DNA detection by droplet digital PCR in non-metastatic operable gastric cancer patients. Cancers 11:396, 2019
29. Coombes RC, Page K, Salari R, et al: Personalized detection of circulating tumor DNA antedates breast cancer metastatic recurrence. Clin Cancer Res 25:4255-4263, 2019
30. Al-Batran S-E, Homann N, Pauligk C, et al: Perioperative chemotherapy with fluorouracil plus leucovorin, oxaliplatin, and docetaxel versus fluorouracil or capecitabine plus cisplatin and epirubicin for locally advanced, resectable gastric or gastro-oesophageal junction adenocarcinoma (FLOT4): A randomised, phase 2/3 trial. Lancet 393:1948-1957, 2019
31. Yang J, Gong Y, Lam VK, et al: Deep sequencing of circulating tumor DNA detects molecular residual disease and predicts recurrence in gastric cancer. Cell Death Dis 11:346, 2020
32. Kasi PM, Fehringer G, Taniguchi H, et al: Impact of circulating tumor DNA-based detection of molecular residual disease on the conduct and design of clinical trials for solid tumors. JCO Precis Oncol 6:e2100181, 2022
33. National Library of Medicine: Testing the Addition of Radiotherapy to the Usual Treatment (Chemotherapy) for Patients With Esophageal and Gastric Cancer That Has Spread to a Limited Number of Other Places in the Body. https://clinicaltrials.gov/ct2/show/NCT04248452
34. Malla M, Loree JM, Kasi PM, Parikh AR: Using circulating tumor DNA in colorectal cancer: Current and evolving practices. J Clin Oncol 40:2846-2857, 2022

APPENDIX

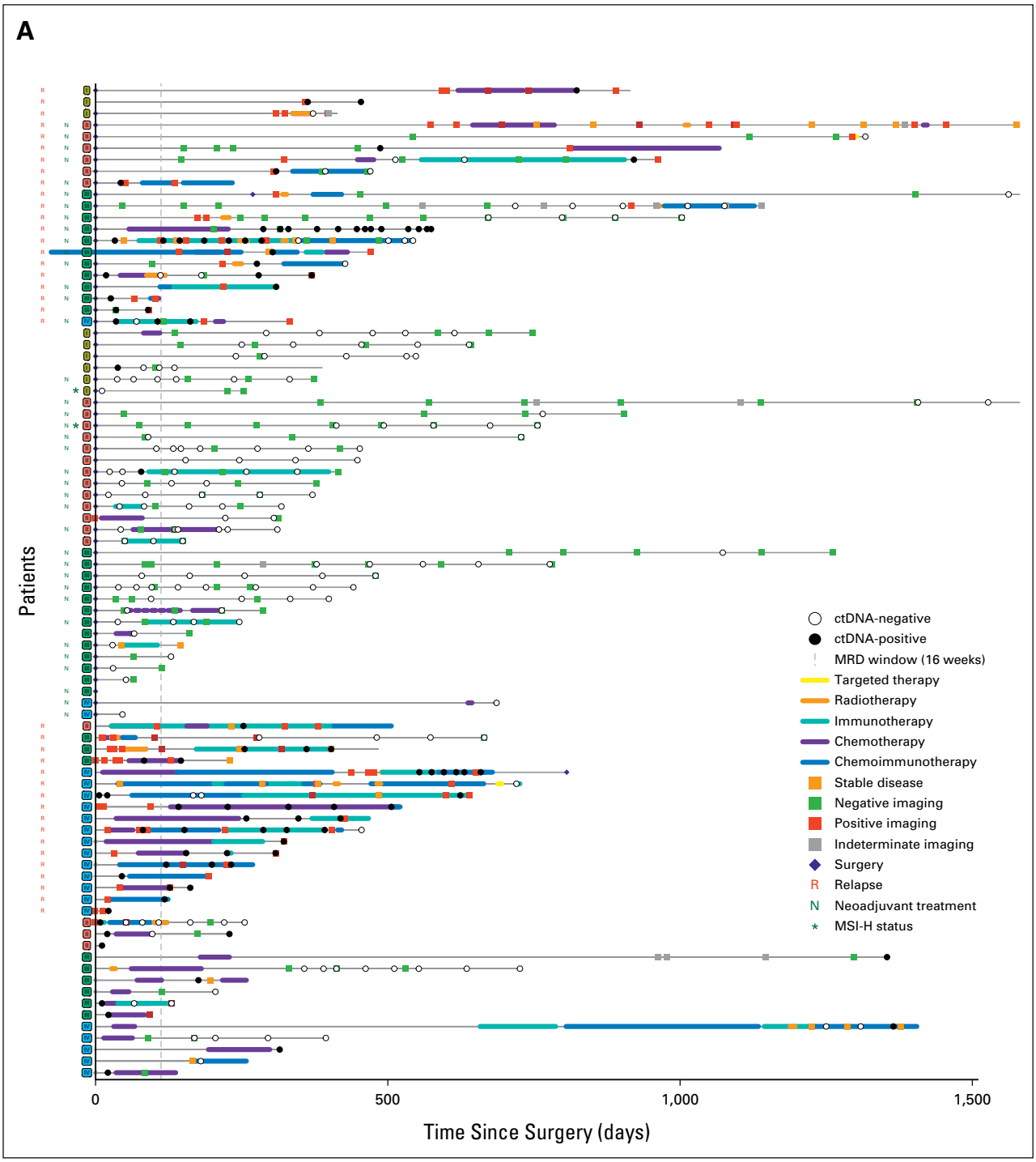

FIG A1. Overview plots depicting the complete clinical course of all patients with (A) esophageal, (B) gastroesophageal junction, and (C) gastric cancers, including results of longitudinal ctDNA analysis. ctDNA, circulating tumor DNA; MRD, molecular residual disease; MSI-H, microsatellite instability high (continued on following page)

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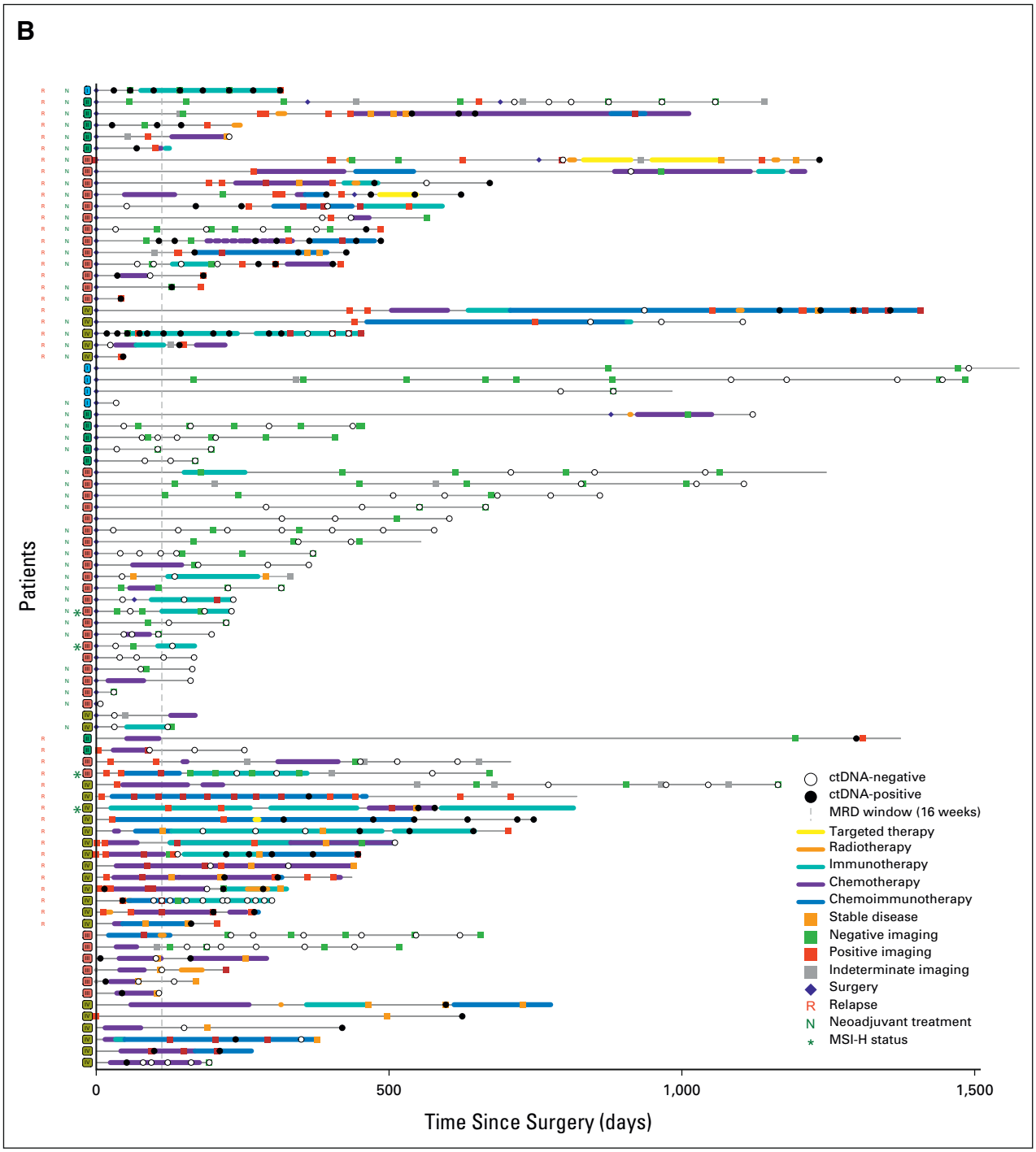

FIG A1. (Continued)

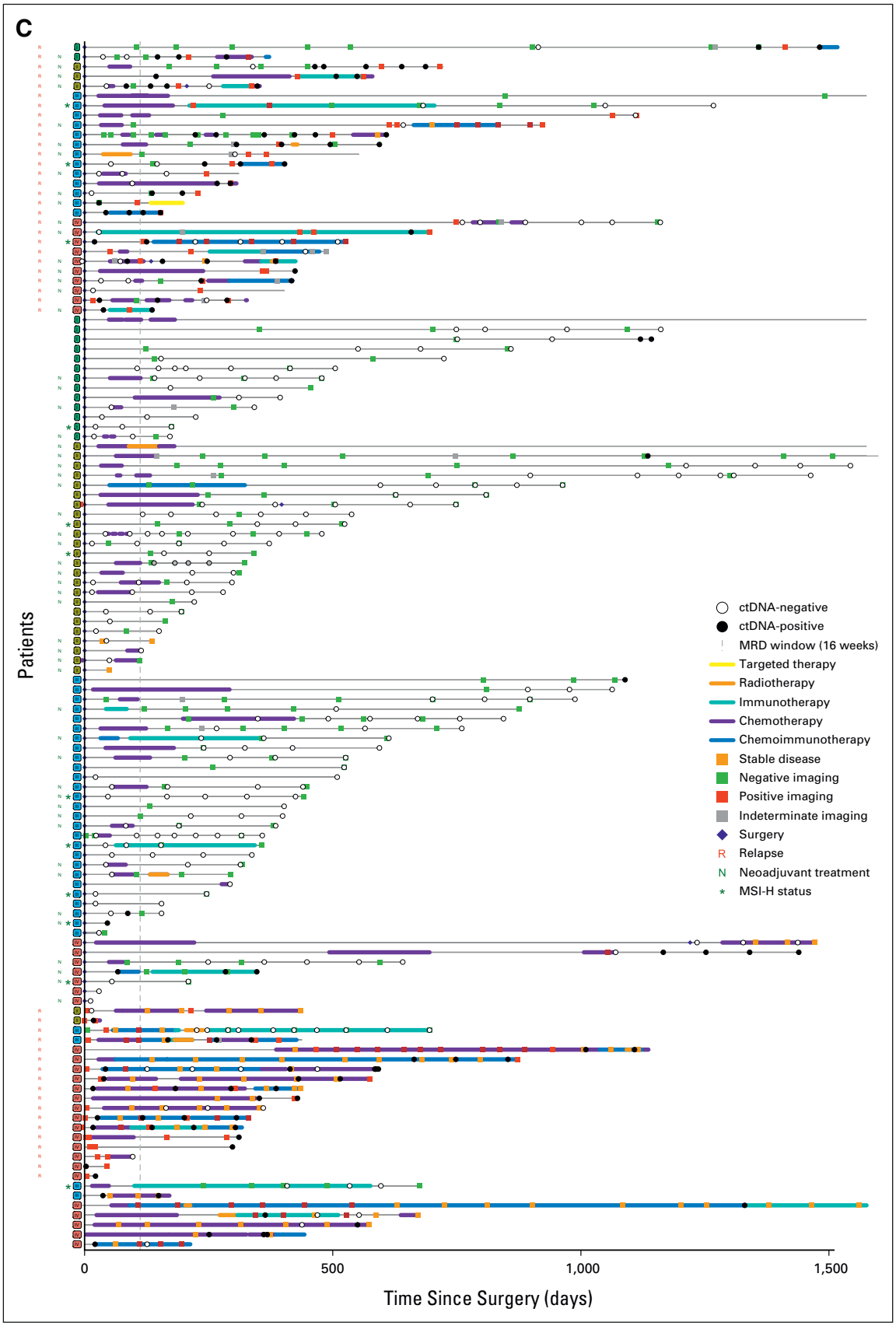

FIG A1. (Continued)

TABLE A1. Cohort Demographics

Clinical Demographic	N = 295, No. (%)
Sex	
Female	99 (33.6)
Male	196 (66.4)
Cancer location	
Esophageal	86 (29.2)
GEJ	85 (28.8)
Gastric	124 (42.0)
Surgery performed	
Yes	210 (71.2)
No	85 (28.8)
Received neoadjuvant treatment	
Yes	138 (46.8)
No	157 (53.2)
Overall stage[a]	
I	29 (9.8)
II	64 (21.7)
III	119 (40.3)
IV	83 (28.1)
Histologic subtype	
Adenocarcinoma	273 (92.5)
Small cell	1 (0.3)
Squamous	21 (7.1)
Histologic grade	
G1	8 (2.7)
G2	50 (17.0)
G3	103 (34.9)
NA	134 (45.4)
Signet ring cells	
Yes	72 (24.4)
No	78 (26.4)
NA	145 (49.2)
HER2 status	
Positive	34 (11.5)
Negative	191 (64.8)
NA	70 (23.7)
PD-L1 CPS	
0	36 (12.2)
1	21 (7.1)
> 1	104 (35.3)
NA	134 (45.4)
MSI status[b]	
MSI-H	18 (6.1)
MSS	274 (92.9)
NA	3 (1.0)

(Continued in next column)

TABLE A1. Cohort Demographics (Continued)

Abbreviations: CPS, combined positive score; G, grade; GEJ, gastroesophageal junction; HER2, human epidermal growth factor receptor 2; MSI, microsatellite instability; MSI-H, microsatellite instability high; MSS, microsatellite stable; NA, not available; PD-L1, programmed cell death ligand 1.

[a]The clinical stage group was used if the patient received neoadjuvant treatment, otherwise the pathologic stage group was used.

[b]MSI status was determined from whole-exome sequencing of tumor tissue using the MANTIS tool. MSI-high cases were more prevalent in the gastric cohort (12/124 = 9.7%) compared with GEJ (4/85 = 4.7%) and esophageal (2/86 = 2.3%) cohorts, but this difference was not statistically significant (P = .06 by chi-squared test).