Supplementary File

Title: Longitudinal circulating tumor DNA analysis during treatment of locally advanced resectable gastric or gastroesophageal junction adenocarcinoma: the PLAGAST prospective biomarker study

Short running title: ctDNA analysis in PLAGAST biomarker study

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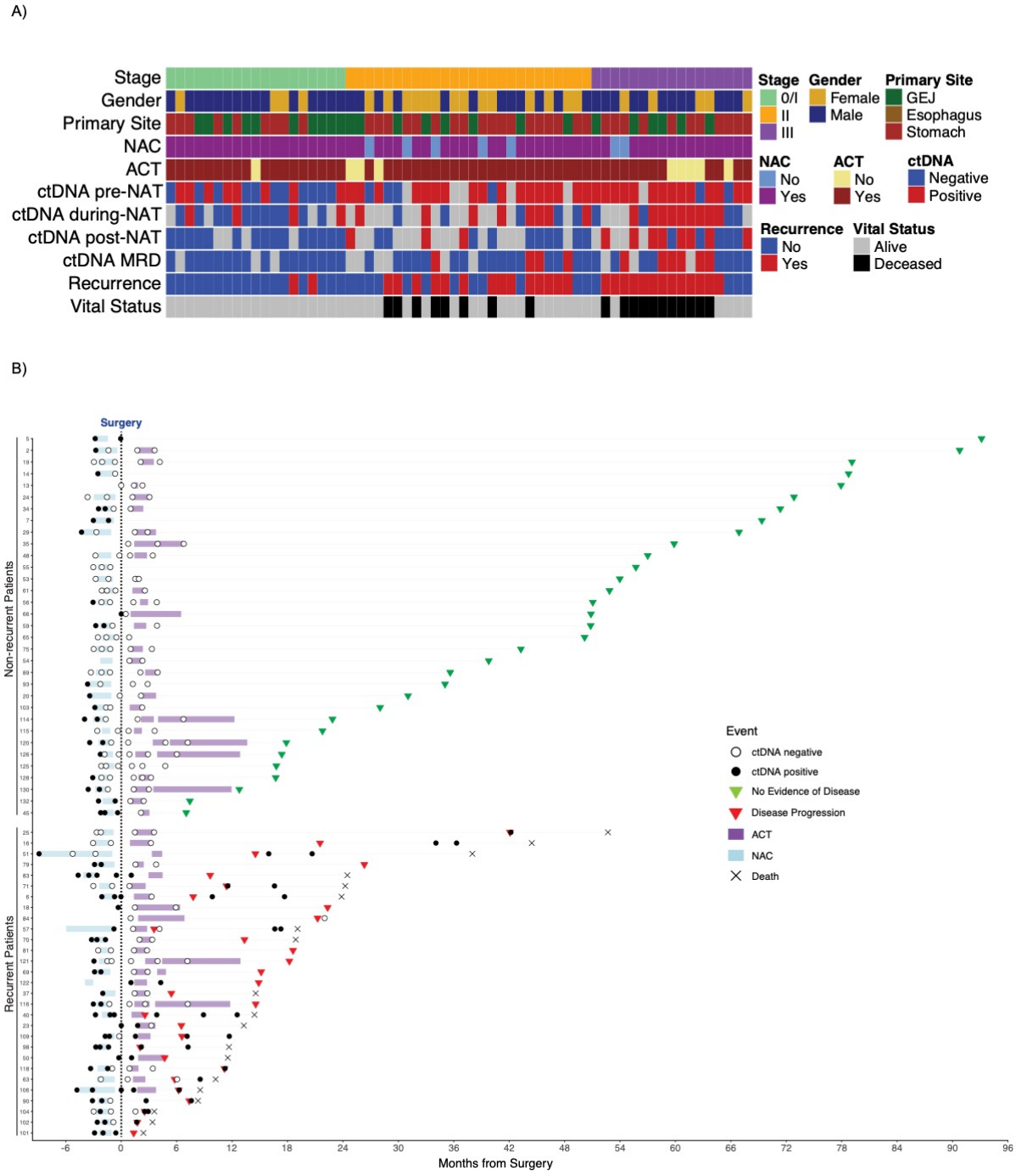
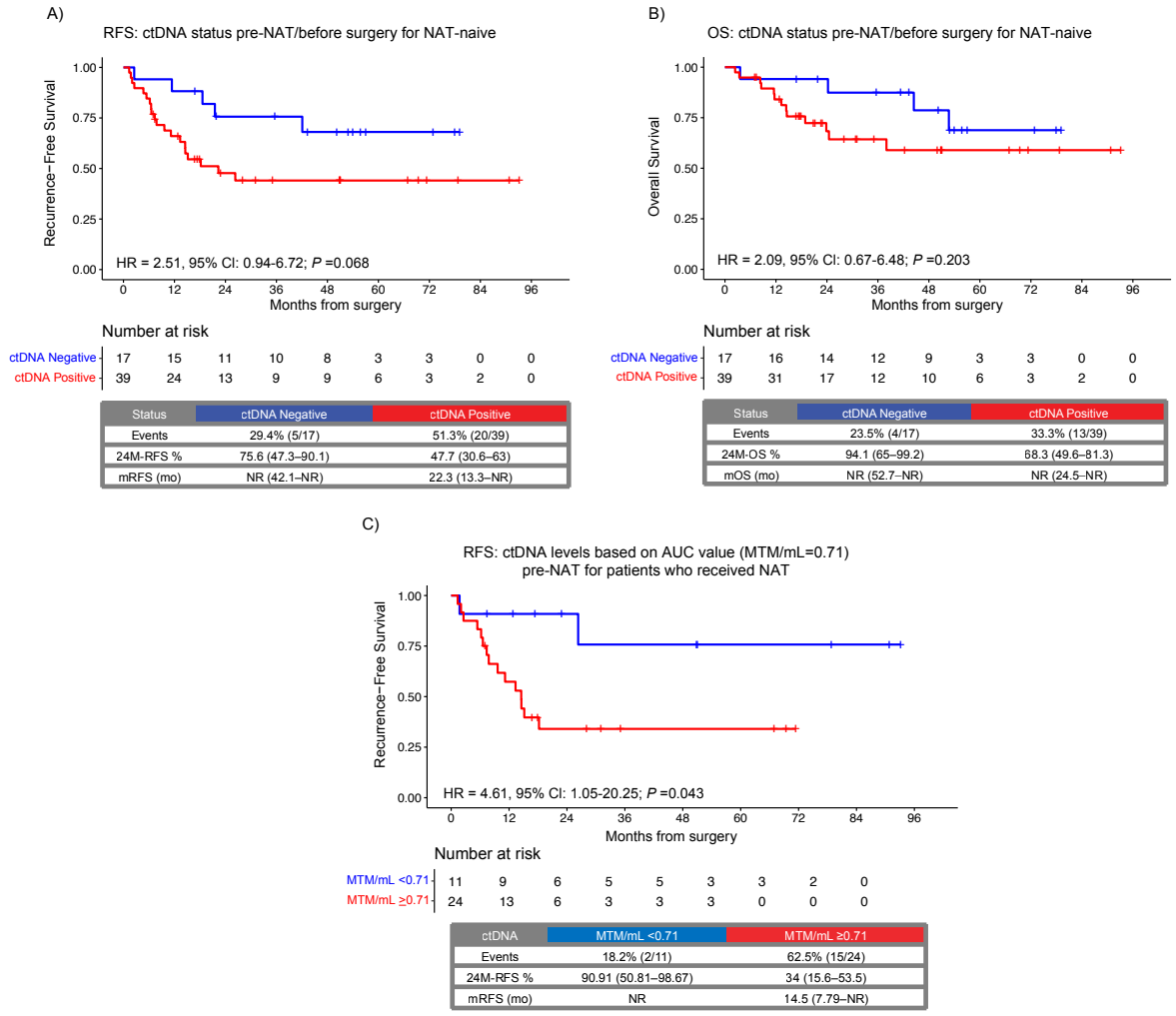
Supplementary Figure 1: Cohort heat map and overview plot



Supplementary Figure 1. Cohort heat map and overview plot. A) Heat map showing clinicopathological and ctDNA detection data for patients. B) Overview plot showing longitudinal ctDNA status, treatment regimen, and clinical outcomes for 62 LAR G/GEJ adenocarcinoma patients. Patients stratified by non-recurrent vs. recurrent cases. Abbreviations: ACT, adjuvant

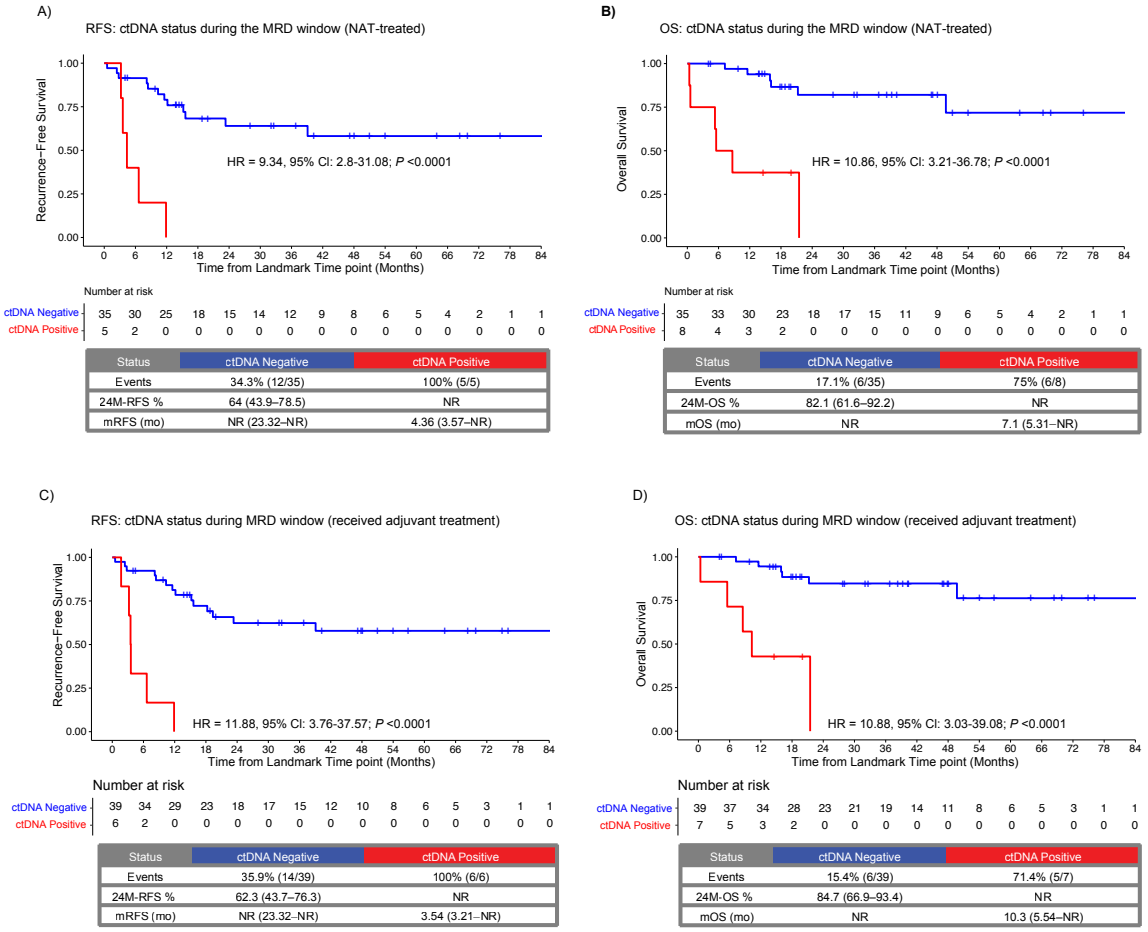
chemotherapy; ctDNA, circulating tumor DNA; MRD, molecular residual disease; NAC, neoadjuvant chemotherapy; NAT, neoadjuvant therapy.

Supplementary Figure 2: Clinical outcomes by ctDNA status/levels pre-NAT



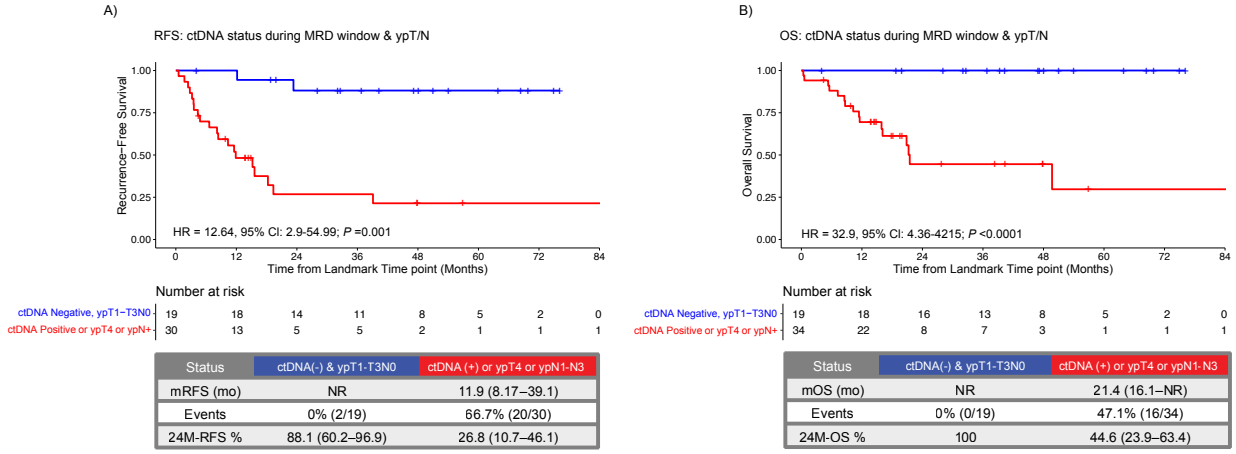
Supplementary Figure 2. **Clinical outcomes by ctDNA status/levels pre-NAT.** Kaplan–Meier estimates of patients with LAR G/GEJ adenocarcinoma representing A) RFS & B) OS stratified by ctDNA status (negative or positive) at pre-NAT/before surgery for NAT naive. C) RFS stratified by MTM/mL based on AUC value (MTM/mL= 0.71) at pre-NAT for patients who received NAT. HRs and 95% CIs were calculated using the Cox proportional hazard model; P values were calculated using the two-sided log-rank test. Abbreviations: AUC, area under curve; ctDNA, circulating tumor DNA; CI, Confidence interval; HR, Hazard ratio; NAT, neoadjuvant therapy; NR, not reached; RFS, Recurrence-free survival; OS, Overall survival.

Supplementary Figure 3: Clinical outcomes by ctDNA status within MRD window for patients who received NAT and adjuvant treatment



Supplementary Figure 3. Clinical outcomes by ctDNA status within MRD window for patients who received NAT and adjuvant treatment. Kaplan–Meier estimates of patients with LAR G/GEJ adenocarcinoma representing A, C) RFS & B, D) OS stratified by ctDNA status (negative or positive) for patients who received NAT (A, B) and those who received adjuvant treatment (C, D). *P* = 0.000272 (A), *P* = 0.000127 (B), *P* = 2.52e-05 (C), *P* = 0.000252 (D). Abbreviations: ctDNA, circulating tumor DNA; CI, Confidence interval; HR, Hazard ratio; MRD, molecular residual disease; NR, not reached; RFS, Recurrence-free survival; OS, Overall survival.

Supplementary Figure 4: Clinical outcomes by ctDNA and ypT/N status



Status	ctDNA(-) & ypT1-T3N0	ctDNA (+) or ypT4 or ypN1-N3
mRFS (mo)	NR	11.9 (8.17–39.1)
Events	0% (2/19)	66.7% (20/30)
24M-RFS %	88.1 (60.2–96.9)	26.8 (10.7–46.1)

Status	ctDNA(-) & ypT1-T3N0	ctDNA (+) or ypT4 or ypN1-N3
mOS (mo)	NR	21.4 (16.1–NR)
Events	0% (0/19)	47.1% (16/34)
24M-OS %	100	44.6 (23.9–63.4)

Supplementary Figure 4. Clinical outcomes by ctDNA and ypT/N status. Kaplan–Meier estimates of patients with LAR G/GEJ adenocarcinoma representing A) RFS & B) OS stratified by ctDNA status within the MRD window and ypT/N stage. *P* = 0.000127 (B). HRs and 95% CIs were calculated using the Cox proportional hazard model. P values were calculated using the two-sided log-rank test. The RFS and OS analyses in the MRD window were landmarked from the date of the MRD time point (12 weeks post-surgery). Median RFS/OS and percentage RFS and OS were estimated from the landmark time point. Abbreviations: ctDNA, circulating tumor DNA; CI, Confidence interval; HR, Hazard ratio; MRD, molecular residual disease; NR, not reached; RFS, Recurrence-free survival; OS, Overall survival.