Personalized Circulating Tumor DNA Analysis to Monitor Colorectal Cancer

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Abstract Number 1590

Introduction

- Early detection of disease recurrence has been shown to improve survival in patients with colorectal cancer (CRC)[1]; detection of circulating tumor DNA (ctDNA) post-operatively defines a subset of CRC patients with very high risk of recurrence.[2,3]
- Previous studies have performed ctDNA analysis to monitor tumor burden in CRC using small gene panel sequencing or digital droplet PCR.[2,4]

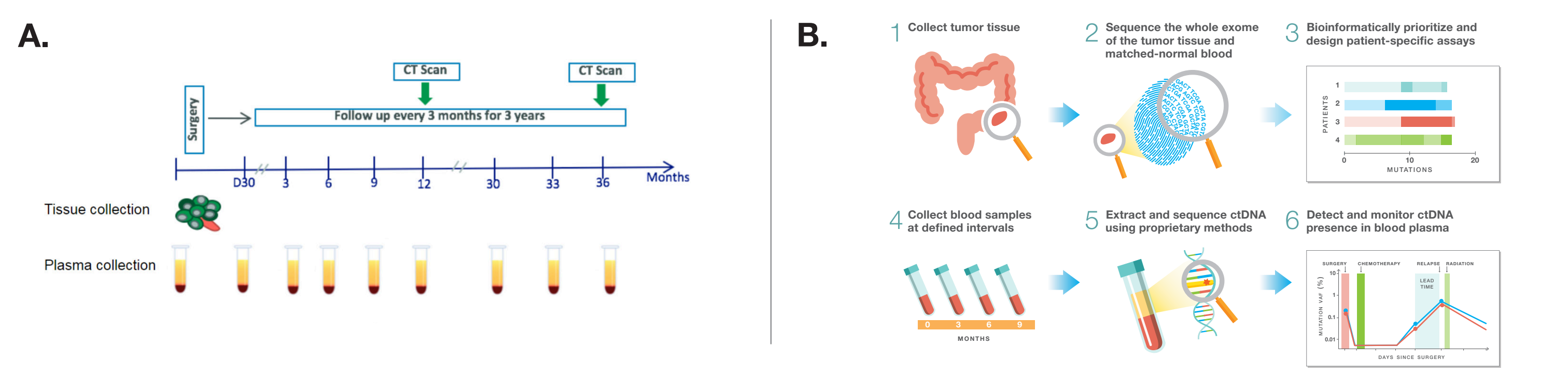
Objectives

- The aim of this study was to use a personalized multiplex-PCR NGS platform targeting 16 tumor-specific mutations per patient to assess minimal residual disease post-operatively and to monitor treatment response in CRC.

Methods

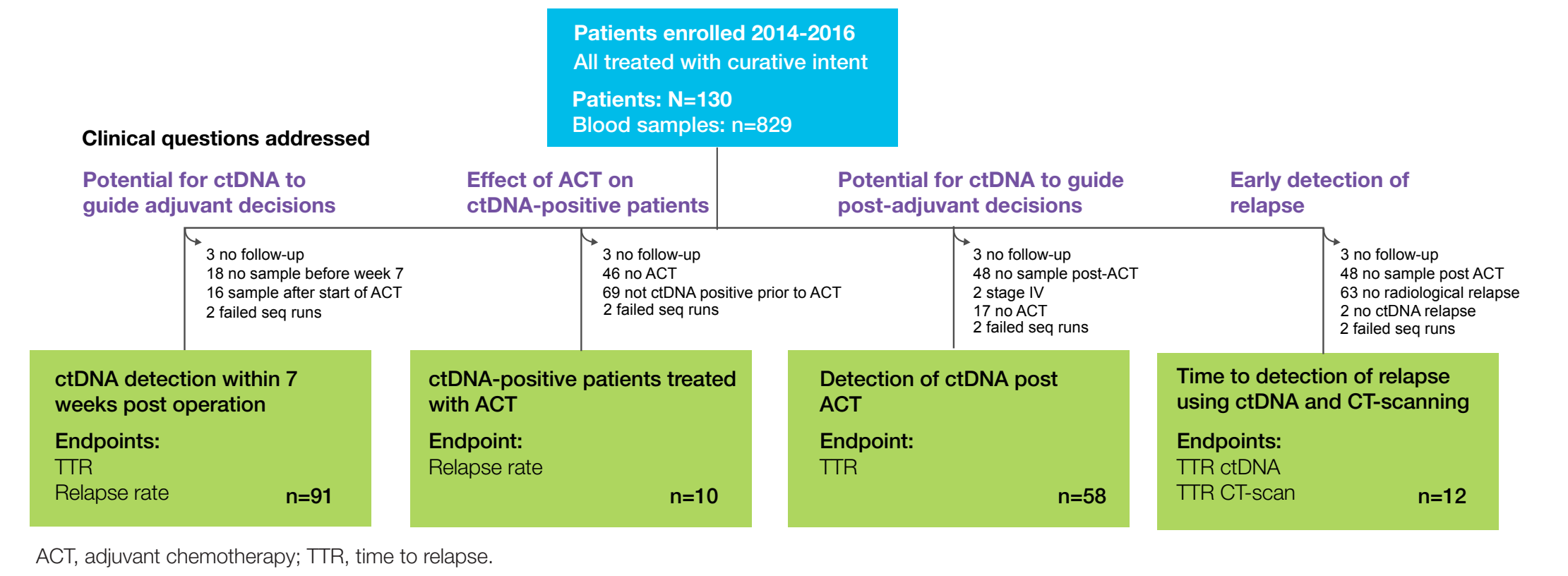
- A cohort of 130 patients with stage I-IV CRC, treated with curative surgery, and (optional) adjuvant chemotherapy (ACT) was included. Plasma samples were collected longitudinally at baseline prior to surgery and at scheduled control visits after surgery (**Figure 1A**).
- Whole-exome sequencing identified somatic mutations; following the analytically-validated Signatera™ workflow (see Abstract Number 4542), patient-specific multiplex-PCR assays targeting 16 somatic single-nucleotide and indel variants were assayed by massively parallel sequencing in plasma collected pre- and post-surgery, and during ACT (**Figure 1B**).

Figure 1. Schematic of Clinical and Molecular Protocols



Results

Figure 2. Study Overview



Patients enrolled 2014-2016
All treated with curative intent
Patients: N=130
Blood samples: n=829

Clinical questions addressed

Potential for ctDNA to guide adjuvant decisions	Effect of ACT on ctDNA-positive patients	Potential for ctDNA to guide post-adjuvant decisions	Early detection of relapse
3 no follow-up 18 no sample before week 7 16 sample after start of ACT 2 failed seq runs	3 no follow-up 46 no ACT 69 not ctDNA positive prior to ACT 2 failed seq runs	3 no follow-up 48 no sample post-ACT 2 stage IV 17 no ACT 2 failed seq runs	3 no follow-up 48 no sample post ACT 63 no radiological relapse 2 no ctDNA relapse 2 failed seq runs
ctDNA detection within 7 weeks post operation **Endpoints:** TTR Relapse rate **n=91**	**ctDNA-positive patients treated with ACT** **Endpoint:** Relapse rate **n=10**	**Detection of ctDNA post ACT** **Endpoint:** TTR **n=58**	**Time to detection of relapse using ctDNA and CT-scanning** **Endpoints:** TTR ctDNA TTR CT-scan **n=12**

ACT, adjuvant chemotherapy; TTR, time to relapse.

Table 1. Patient Characteristics and Demographics (N=130)

Patients, N = 130, Age (years), median (range) = 69.9 (43.3-91), Imaging follow-up, months, median = 12.3

Gender, n (%)		**Histological grade, n (%)**	
Female	56 (43.1)	Moderatley differentiated	100 (76.9)
Male	74 (56.9)	Poorly differentiated	20 (15.5)
		ND	10 (7.7)
Location, n (%)		**Adjuvant treatment, n (%)**	
		I	0 (0)
Colon	124 (95.4)	II	6 (14.3)
Rectum	6 (4.6)	III	71 (88.8)
		IV	2 (100)
		Total	79 (60.8)
Pathological stage, n (%)		**Relapse, n (%)**	
I	6 (4.7)	I	0 (0)
II	42 (3.2)	II	3 (7.1)
III	80 (62.5)	III	19 (23.8)
IV	2 (1.6)	IV	0 (0)
		Total	22 (16.9)
Histological type, n (%)		**MSS/MSI**	
Adenocarcinoma	120 (92.3)	MSS	104 (80)
Mucinous carcinoma	10 (7.7)	MSI	17 (13.1)
		ND	9 (6.9)

ND, not determined.

Figure 3. Patient Summaries for 36 Months of Surveillance and Plasma Collection

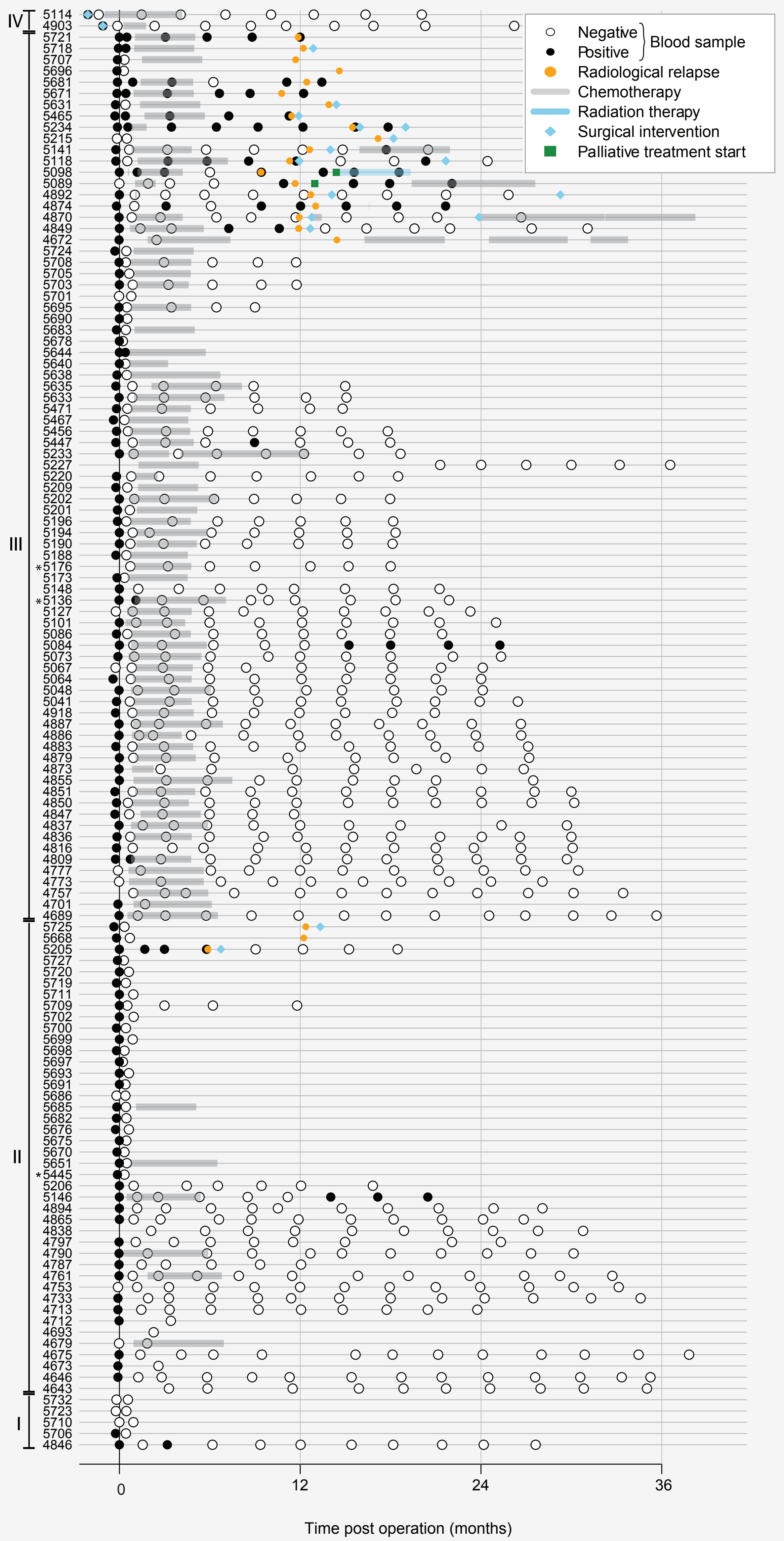


Figure 3. Schematic overview of ctDNA profiling results of more than 800 plasma samples from 128 of 130 patients. The initial sequencing runs failed for the two remaining patients and are currently being rerun.

Figure 4. Relapse Risk Stratified by Post-Operative ctDNA Status

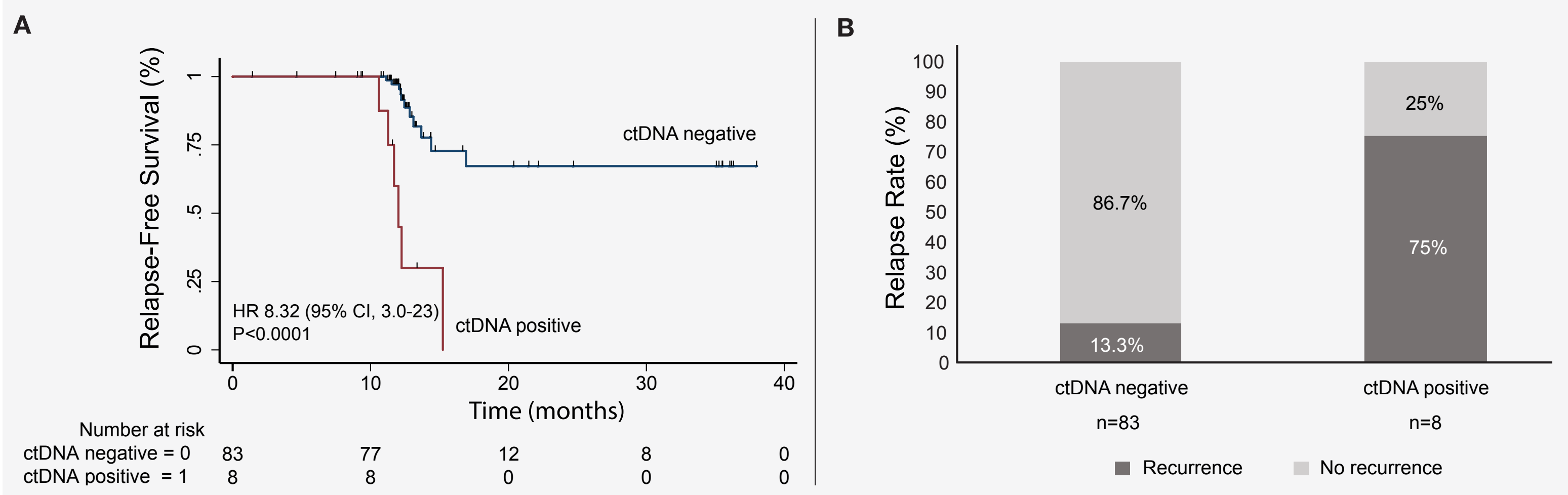


Figure 4. ctDNA status was established based on the first post-operative blood sample, which was drawn by week 6 and prior to start of ACT. (A) Kaplan Meier analysis of recurrence free survival stratified by ctDNA status. Patients without event were censored at end of follow-up. (B) Relapse rates according to ctDNA status (no patients censored). ACT was given to 58 patients, which likely affected the relapse rate of ctDNA-positive patients (**Figure 6** shows how ctDNA was cleared by ACT for the two non-relapsing ctDNA positive patients).

Figure 5. Post-Therapy Relapse Risk Stratified by Post-operative ctDNA Status

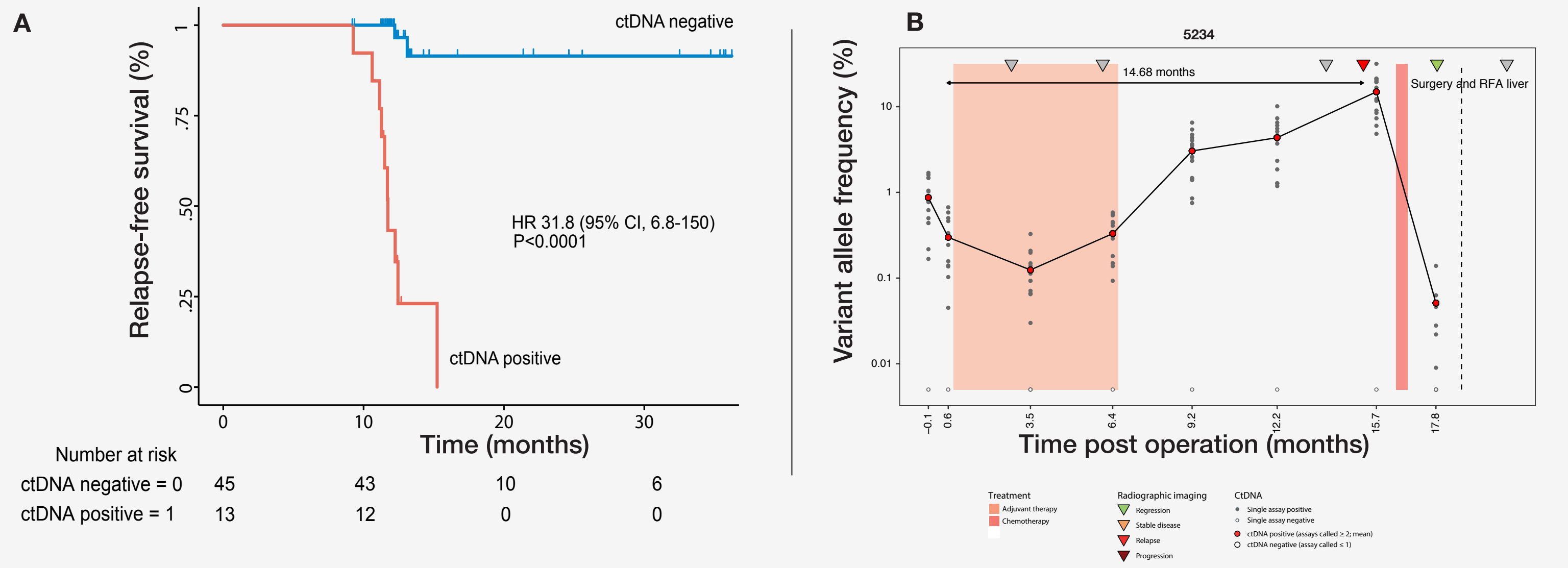


Figure 5. (A) Relapse risk post-ACT, stratified by post-adjuvant ctDNA status. Patients were scored ctDNA positive if any timepoint post-adjuvant was positive and negative if all post-adjuvant timepoints were negative. (B) ctDNA profiling of a representative patient during adjuvant and post-adjuvant treatment.

Figure 6. Effectiveness of Adjuvant Therapy in Preventing Relapse

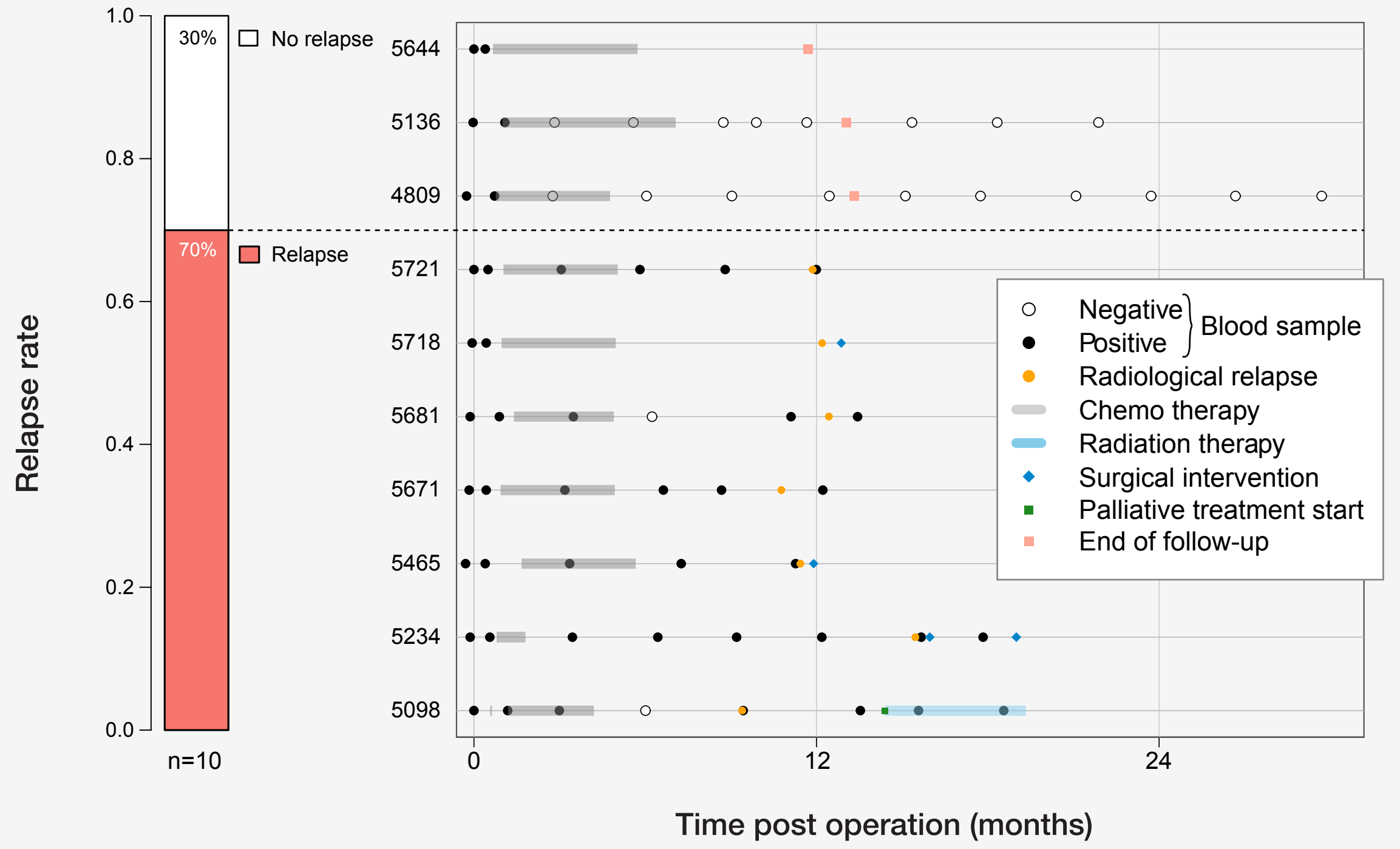


Figure 6. Relapse rate of 10 patients post-operative ctDNA-positive prior to treatment with ACT.

Figure 7. Time to Relapse (TTR) Based on Radiology and ctDNA

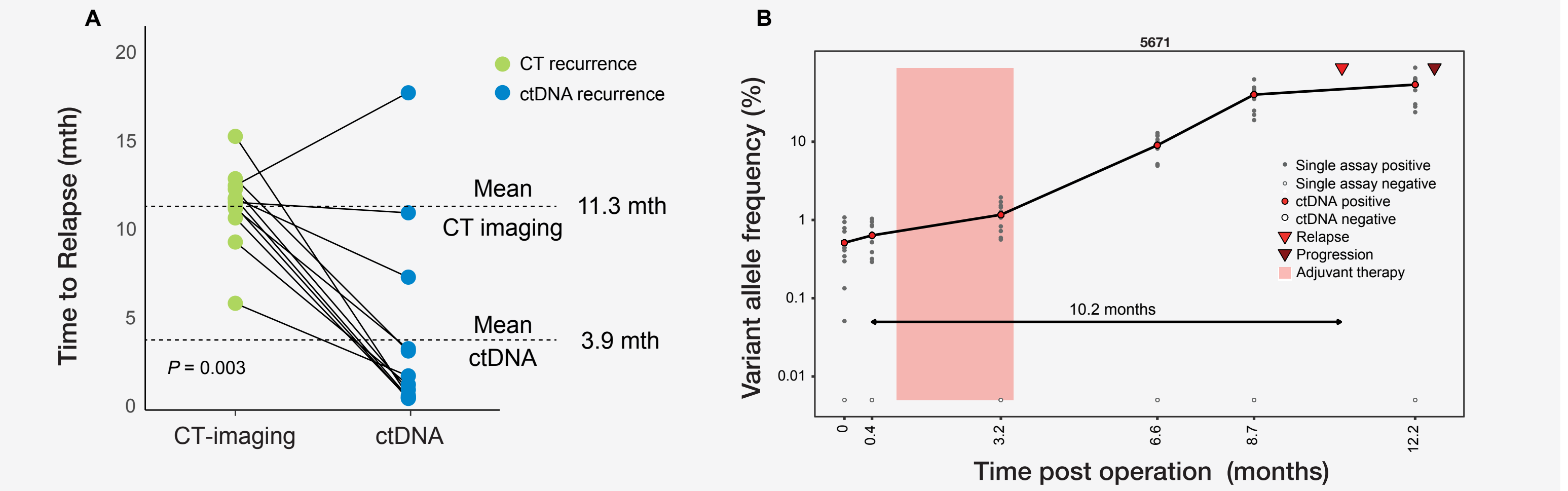


Figure 7. (A) Comparison of TTR using ctDNA and CT-imaging for the 12 recurrence patients with recurrence was detected by both modalities. (B) Serial ctDNA profiling of a representative recurrence patient with a 10.2 months ctDNA lead-time.

Figure 8. Early Detection of Relapse and Prediction of Treatment Response

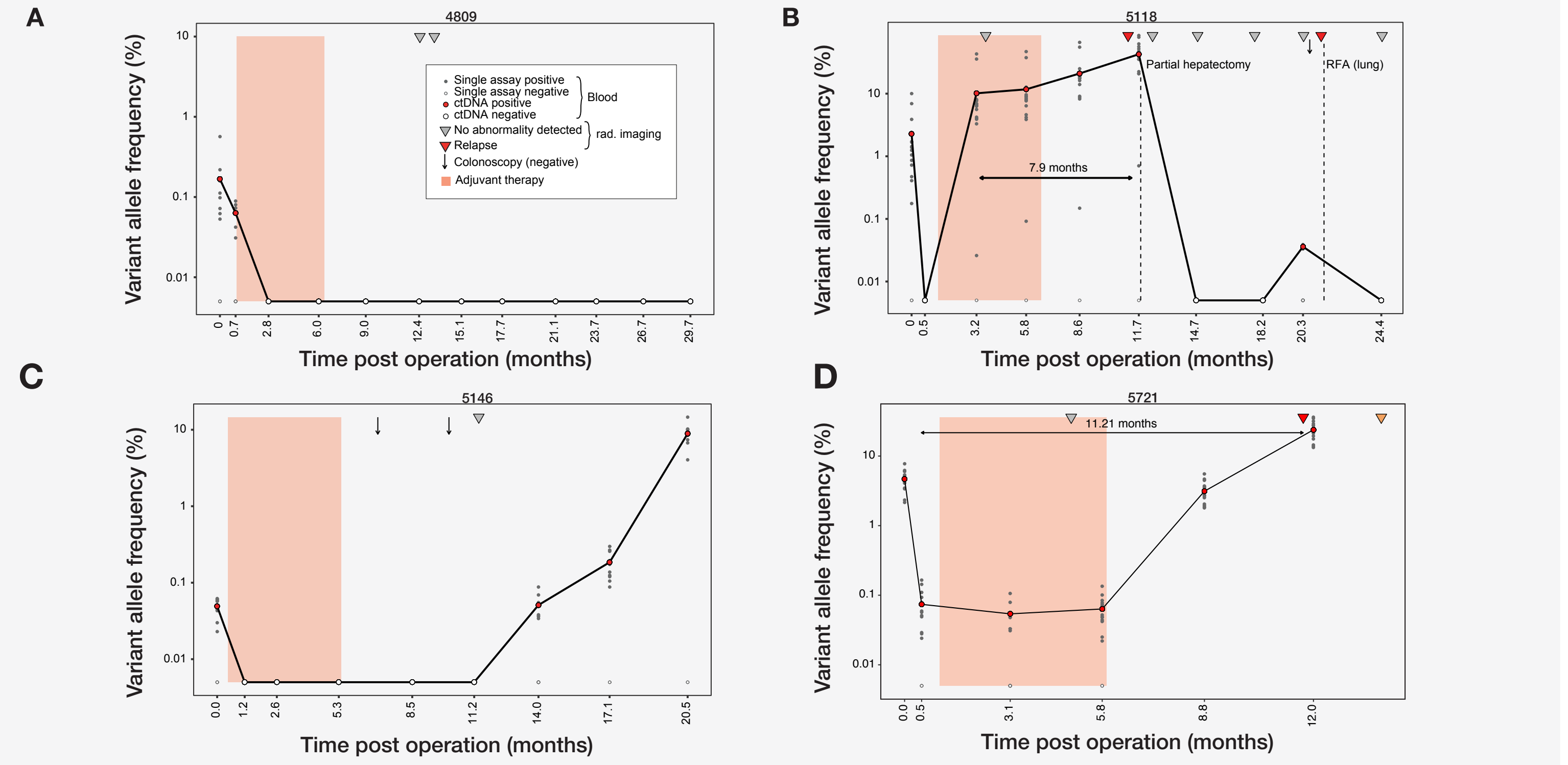


Figure 8. Serial ctDNA profiling of four represenatative patients.

Conclusions

- The Signatera RUO approach for massively parallel sequencing of personalized multiplex-PCR assays targeting tumor specific mutations is a highly sensitive and specific platform for detection and quantification of ctDNA.
- Post-operative ctDNA analysis enables stratification of CRC patients into subgroups with either very high or very low recurrence risk, both prior to and after ACT.
- Longitudinal ctDNA analysis enables efficient post-operative treatment monitoring and early detection of recurrence.
- ctDNA analysis has great potential to guide treatment decisions, both in the adjuvant and post-adjuvant setting.

References

1. Pita-Fernàndes, et al. Ann Oncol. 2015. 26(4):644-56. 2. Tie J, et al. Sci Transl Med. 2016. 8(346):346ra92. 3. Schøler LV, et al. Clin Cancer Res. 2017. 23(18):5437-45. 4. Reinert T, et al. Gut. 2016. 65(4):625-34.

American Association for Cancer Research Annual Meeting 2018; Chicago, IL | April 14–18, 2018