

# Longitudinal Analysis of Cell-free Mutated KRAS and CA 19-9 Predicts Survival Following Curative Resection of Pancreatic Cancer

**Saskia Hussung**

UniversitätsSpital Zurich <https://orcid.org/0000-0001-7987-0065>

**Dilara Akhoundova**

UniversitätsSpital Zurich

**Julian Hipp**

Universitätsklinikum Freiburg

**Marie Follo**

Universitätsklinikum Freiburg

**Rhena Klar**

Universitätsklinikum Freiburg

**Ulrike Philipp**

Universitätsklinikum Freiburg

**Florian Scherer**

Universitätsklinikum Freiburg

**Nikolas von Bubnoff**

Universitätsklinikum Freiburg

**Justus Duyster**

Universitätsklinikum Freiburg

**Melanie Börries**

Albert-Ludwigs-Universität Freiburg

**Uwe Wittel**

Universitätsklinikum Freiburg

**Ralph Fritsch** (✉ [Ralph.Fritsch@usz.ch](mailto:Ralph.Fritsch@usz.ch))

UniversitätsSpital Zurich

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## Research article

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# Abstract

**Background:** Novel biomarkers and molecular monitoring tools hold potential to improve outcome for patients following resection of pancreatic ductal adenocarcinoma (PDAC). We hypothesized that the combined longitudinal analysis of mutated cell-free plasma *KRAS* (cf*KRAS*<sup>mut</sup>) and CA19-9 during adjuvant treatment and follow-up might more accurately predict disease course than hitherto available parameters.

**Methods:** Between 07/2015 and 10/2018, we collected 134 plasma samples from 25 patients (pts) after R0/R1-resection of PDAC during adjuvant chemotherapy and post-treatment surveillance at our institution. Highly sensitive discriminatory multi-target ddPCR assays were employed to screen plasma samples for cf*KRAS*<sup>mut</sup>. cf*KRAS*<sup>mut</sup> and CA19-9 dynamics were correlated with recurrence-free survival (RFS) and overall survival (OS). Patients were followed-up until 01/2020.

**Results:** Out of 25 enrolled patients, 76% had undergone R0 resection and 48% of resected PDACs were pN0. 17/25 (68%) of patients underwent adjuvant chemotherapy. Median follow-up was 22.0 months, with 19 out of 25 (76%) pts relapsing during study period. Median RFS was 10.0 months, median OS was 22.0 months. Out of clinicopathologic variables, only postoperative CA19-9 levels and administration of adjuvant chemotherapy correlated with survival endpoints. cf*KRAS*<sup>mut</sup> was detected in 12/25 (48%) of patients, and detection of high levels inversely correlated with survival endpoint. Integration of cf*KRAS*<sup>mut</sup> and CA 19-9 levels outperformed either individual marker. cf*KRAS*<sup>mut</sup> outperformed CA19-9 as dynamic marker since increase during adjuvant chemotherapy and follow-up was highly predictive of early relapse and poor OS.

**Conclusions:** Integrated analysis of cf*KRAS*<sup>mut</sup> and CA19-9 levels is a promising approach for molecular monitoring of patients following resection of PDAC. Larger prospective studies are needed to further develop this approach and dissect each marker's specific potential.

## Background

Despite significant progress in understanding tumor genetics and the molecular mechanisms driving tumor development and resistance to therapy, only minor improvements have been achieved to date in the treatment of patients with pancreatic ductal adenocarcinoma (PDAC). With an average 5-year overall survival (OS) rate of only 10% across all stages, most patients still succumb to their disease, making PDAC one of the most aggressive tumor entities [1] [2] [3]. The only potentially curative treatment is surgical resection of early-stage tumors [4] [5]. However, recurrence rates even after R0 resection remain unacceptably high [6] [7] [8] [9] [10]. The integration of more efficacious systemic chemotherapy regimens has improved median overall survival [11], yet responses of individual PDACs to chemotherapy are highly heterogeneous and personalization of perioperative therapy is in its infancy [12] [13] [14] [15] [16] [17] [18]

Consequently, the development and validation of novel biomarkers and molecular monitoring tools to predict disease course and assess efficacy of adjuvant chemotherapy are urgently needed. The analysis of tumor-derived cell-free nucleic acids (ctDNA) extracted from the plasma and other body fluids is a promising tool for molecular diagnostics and non-invasive monitoring of cancer patients [19] [20] [21] [22] [23] [24] [25]. Up to 95% of PDACs harbor activating hot spot mutations in *KRAS* which are readily detectable in the circulation of PDAC patients [20] [26] [27] [28]. We recently described the development and validation of highly sensitive single-target and discriminatory multi-target *KRAS* ddPCR assays for the analysis of cfDNA [29]. These assays allow identification and quantification of mutated *KRAS* directly from circulation without previous knowledge of tumor *KRAS* mutational status, which is not routinely tested for resectable PDACs.

For this study, we hypothesized that longitudinal assessment of cf*KRAS*<sup>mut</sup> following curative resection of PDAC in combination with established protein biomarkers might better identify pts at risk for imminent tumor relapse, indicate failure of adjuvant treatment and ultimately guide treatment according to molecular monitoring. To study the feasibility of this approach, we analyzed plasma samples collected from patients undergoing adjuvant chemotherapy and post-treatment surveillance at our institution in a single-center retrospective biomarker study aiming to identify associations between cf*KRAS*<sup>mut</sup> and CA19-9 dynamics and clinical outcome post PDAC resection.

## Methods

### Study design and population

25 patients were included in a retrospective observational single center biomarker study conducted at Freiburg University Medical Center. Local institutional review board (IRB) approved all study procedures (EK48/18). All patients provided written informed consent for sample collection and analysis. All patients underwent adjuvant chemotherapy and/or post-treatment surveillance at our institution. Inclusion criteria included status post R0 or R1 resection of pancreatic adenocarcinoma with curative intent within 8 weeks of first sample collection and availability of plasma samples for cfDNA extraction. Key exclusion criteria included R2 resection, evidence of metastatic disease on pre- or postoperative CT staging, histologies other than adenocarcinoma. Primary endpoint was detection of cf*KRAS*<sup>mut</sup> in at least one sample during study period. Secondary endpoints included association between changes in cf*KRAS*<sup>mut</sup> and relapse-free survival (RFS) and overall survival (OS). Additionally, clinical, pathologic, treatment- and outcome-related data were analyzed.

### Extraction of cell-free DNA (cfDNA) from plasma samples

Blood samples were collected using commercially available EDTA tubes and plasma was extracted and frozen within one hour of collection. Plasma was extracted through two subsequent centrifugation steps at 3000 rpm and 14000 rpm, each for 10 min at 4 °C. Obtained plasma was stored at -80 °C until extraction of cfDNA. cfDNA was extracted from 4 ml plasma following the SEP/SBS protocol of the PME-

free circulating DNA extraction kit (Analytik Jena, cat. no. 845-IR-0003050), following manufacturer's instructions. Two subsequent elution steps with each 30 µl Elution Buffer were performed to optimize the yield of extracted cfDNA. DNA was stored at -20 °C until cfDNA quantification. cfDNA was evaluated with fragment analyzer and quantified using Qubit 2.0 fluorometer. In patients with resectable PDAC, DNA yield from 4 ml of plasma typically ranged from 1 to 20 ng/µl.

## **Droplet digital PCR (ddPCR)**

ddPCR for cf*KRAS*<sup>mut</sup> was performed as recently described (Hussung et al, 2020). In brief, cfDNA was screened for the presence of the 11 most commonly found *KRAS* hot spot mutations, in PDAC, covering more than 90% of PDAC cases. Highly sensitive single-target assays were used to confirm presence of the mutation identified. Primers and probes were manufactured by Integrated DNA Technologies (IDT, Inc., Coralville, Iowa, USA), sequences and all assay procedures are detailed in Hussung et al [29].

Limit of detection (LOD) and limit of blank (LOB) of the individual assays have been previously described [29].

## **Statistical analysis**

Recurrence-free survival (RFS) was defined as time from resection of PDAC to the first radiologic recurrence (local or distant) or death due to PDAC. Overall survival (OS) was defined as time from the date of diagnosis until death due to any cause. The Kaplan–Meier survival analysis was performed to calculate both RFS and OS. Univariate analyses were performed using the log-rank test. In order to explore independent prognostic factors for RFS and OS, we used backward stepwise Cox regression modeling to estimate hazard ratio (HR) with 95% confidence interval (CI). To compare independent variables, Chi-squared or Fisher's exact test and the Mann–Whitney (rank-sum) test were performed. All statistical analyses were performed using GraphPad Prism Version 5.03 (GraphPad Software, Inc., La Jolla, California, USA) and SPSS 25 software Version 1.0.0.1327 (IBM Corporation, New York, United States). *P* values < 0.05 were considered as statistically significant.

## **Results**

### ***Patient cohort***

25 patients with nonmetastatic, R0/R1-resected adenocarcinoma of the pancreas were included in the study. Patient characteristics are summarized in Table 1. R0 resection rate was 76% (19/25), 12/25 (48%) of tumors were nodal negative (pN0). 17/25 (68%) patients underwent adjuvant chemotherapy. Median follow-up for the cohort was 22.0 months, with 19 out of 25 (76%) pts relapsing during this period. Median RFS for the cohort was 10.0 months, median OS was 22.0 months. We performed univariate and multivariate survival analyses (Supplemental Tables 1 and 2, Figure S1) for established clinicopathologic variables and found a trend towards inferior RFS but not OS for R1 resection (Figure S1 A,B), a significant inverse correlation between elevated CA19-9 in the first sample collected after resection and RFS and OS

(Figure S1 C,D) and significantly better OS for patients undergoing adjuvant chemotherapy (Figure S1 E,F).

Table 1  
Patient and tumor characteristics

<b>Clinicopathologic features</b>	n =25 (%)
<b>Median age (years)</b>	75
<b>Age range</b>	42 – 81
<b>Sex</b>	
<b>Male</b>	18 (72)
<b>Female</b>	7 (28)
<b>Tumor location</b>	
<b>Pancreas head</b>	20 (80)
<b>Pancreas body &amp; tail</b>	5 (20)
<b>T stage</b>	
<b>T1 – T2</b>	7 (28)
<b>T3</b>	18 (72)
<b>N status</b>	
<b>N0</b>	12 (48)
<b>N1-2</b>	13 (52)
<b>R status</b>	
<b>R0</b>	19 (76)
<b>R1</b>	5 (20)
<b>Rx</b>	1 (4)
<b>Lymphovascular invasion</b>	
<b>L0</b>	15 (60)
<b>L1</b>	10 (40)
<b>Perineural invasion</b>	
<b>Pn0</b>	2 (8)
<b>Pn1</b>	23 (92)
<b>Grading</b>	
<b>G2</b>	13 (52)
<b>G3</b>	12 (48)

<b>Adjuvant chemotherapy</b>		
<b>Yes</b>	<b>1</b>	<b>7 (68)</b>
<b>No</b>		<b>8 (32)</b>
<b>Time to relapse (months)</b>		
<b>Median</b>		<b>10</b>
<b>Range</b>		<b>0.5 – 42</b>
<b>Overall survival (months)</b>		
<b>Median</b>		<b>22</b>
<b>Range</b>		<b>0.5 - 46</b>

## ***Analysis of plasma cfKRAS<sup>mut</sup>***

We analyzed 134 plasma samples collected from 25 patients at routine follow-ups before, during and after adjuvant chemotherapy. First samples were taken at a median of 40 days (95% CI 26-50) after resection prior to adjuvant chemotherapy. Median number of samples collected was 4 samples per patient (95% CI 3-5 samples). Median time interval between sampling was 70 days (95% CI 63-91). We screened cfDNA extracted from plasma samples for the presence of cfKRAS<sup>mut</sup> with recently described discriminatory multi-target KRAS ddPCR assays, covering the 11 most common KRAS hot spot mutations in PDAC [29]. At the postoperative stage, no molecular pathology data was available for any tumor. However, for a subset of patients KRAS mutational status became available at relapse (Supplemental Table 3).

Across all samples analyzed, cfKRAS<sup>mut</sup> was detected in 34/134 (25%) samples and 12/25 (48%) of patients for at least one time point. In 16/16 (100%) patients with later on determined tumor tissue KRAS mutational status, the SNV detected by ddPCR in plasma (cfKRAS<sup>mut</sup>) matched the KRAS SNV detected in tissue analysis (Supplemental Table 3), confirming the validity of ddPCR cfKRAS<sup>mut</sup> analysis. In 0/134 (0%) plasma samples more than one KRAS SNV could be detected above assay threshold.

## ***Association of cfKRAS<sup>mut</sup> and elevated CA19-9 levels with survival endpoints***

Detection of cfKRAS<sup>mut</sup> at any time point during study course above assay threshold was not associated with RFS or OS (Figure 1 A, B). However, when a threshold of 15 copies KRAS<sup>mut</sup> per ml plasma for cfKRAS<sup>mut</sup> was chosen, cfKRAS<sup>mut</sup> positivity at any time point during study period was strongly associated with early relapse and poor survival (Figure 1 C, D). CA19-9 levels were determined from the



same blood collections as part of clinical assessment. 12/25 (48%) of patients had at least one blood sample with CA19-9 above normal range during study course. Increased CA19-9 at any time point was associated with significantly inferior RFS and a trend towards inferior OS (Figure 1 E, F). Notably, only 6/12 (50%) patients were double positive for cfKRAS<sup>mut</sup> and CA19-9, indicating that cfKRAS<sup>mut</sup> and CA19-9 positivity are not redundant. Patients with either CA19-9 positivity or cfKRAS<sup>mut</sup> levels > 15 copies/mL cfKRAS during study course (14/25, 56%) showed inferior RFS and OS, indicating that the integration of both biomarkers might be predictive and prognostic for a larger group of patients than assaying them individually (Figure 1 G, H). Survival of double positive patients was similar to single positive patients in our cohort (data not shown).

## ***Association of cfKRAS<sup>mut</sup> and CA19-9 dynamics with survival***

Protein tumor markers and cfDNA are highly dynamic biomarkers for the molecular monitoring of disease course and treatment response. We therefore next analyzed whether changes over time in either biomarker are associated with outcome in our cohort. For each 9/18 (50%) patients with a sufficient number of follow-up samples, cfKRAS<sup>mut</sup> or CA19-9 levels increased during observation period. Increase of cfKRAS<sup>mut</sup> was associated with significantly reduced OS (Figure 2 A), while increase of CA19-9 was associated with a non-significant trend towards inferior OS (Figure 2 B). Similarly, early increase of cfKRAS<sup>mut</sup>, defined as increase within 6 months after surgery, was strongly associated with inferior OS while early CA19-9 increase was associated with only a trend towards shorter OS (Figure 2 C, D). Integrating both markers for the analysis of dynamic changes over time did not outperform cfKRAS<sup>mut</sup> alone (Figure 2), suggesting that cfKRAS<sup>mut</sup> might be the biomarker of choice for longitudinal monitoring in this setting.

## ***Single patient analysis***

Figure 3 illustrates the relationship between cfKRAS<sup>mut</sup> and CA19-9 (Figure 3A) dynamics and tumor relapse for individual patients. 13/18 patients in the analysis relapsed during observation period. Increase of cfKRAS<sup>mut</sup> or CA19-9 was significantly associated with relapse. Single-patient analysis also illustrates dynamic changes during adjuvant chemotherapy and follow-up with several patients showing transient increases followed by decreases of either marker.

Figure 3B illustrates that in most patients, relapsed was preceded by a strong increase of CA19-9 or cfKRAS<sup>mut</sup>. However, single patient analyses also illustrate that both cfKRAS<sup>mut</sup> and CA19-9 are both highly dynamic markers and that individual patterns are highly heterogenous. Larger cohorts and prospective trials are required to better unravel the relationships and the temporal relationship between biomarker dynamics and clinical relapse and to study the impact of therapeutic intervention.

## Discussion

In an exploratory analysis, we followed a small cohort of pancreatic cancer patients after curative resection of pancreatic adenocarcinoma through adjuvant therapy and post-treatment follow-up. We analyzed mutated *KRAS* in cell-free DNA with discriminatory ddPCR assays and integrated results with CA19-9 levels for association with relapse and survival endpoints. Numerous studies have unveiled the potential of the analysis of cell-free mutated tumor DNA as novel diagnostic [26], predictive [30] [31] [32] and prognostic [30] [31] [33] [34] [35] [36] biomarker for pancreatic cancer.

What takes our study apart is the use of discriminatory multi-target *KRAS* ddPCR assays [29] to directly identify *KRAS* SNVs without performing previous tumor NGS. These assays have higher sensitivity compared to many available NGS-based assays (Hussung et al). In comparison to more sophisticated NGS panels specifically developed for cfDNA analysis [28] [37], multi-target ddPCR assays are associated with much lower assay costs, allowing for the serial analysis through clinical course analogous to CA19-9 levels. Using these assays, our cf*KRAS*<sup>mut</sup> detection rate in the cohort was similar to other published data for patients following PDAC resection [35] [30] [20]. A 100% concordance rate between tumor tissue and detected cfDNA *KRAS* SNVs further validates our approach.

In our cohort, detection of cf*KRAS*<sup>mut</sup> in the first postoperative sample alone did not significantly correlate with survival (data not shown), while elevated CA19-9 levels at first presentation were associated with poor outcome. Similarly, positivity for cf*KRAS*<sup>mut</sup> at any time point above assay threshold alone was not significantly associated with survival. However, when choosing a more stringent cf*KRAS*<sup>mut</sup> cut-off or when analyzing dynamic changes (increase vs non-increase), cf*KRAS*<sup>mut</sup> was strongly associated with survival and outperformed CA19-9 levels for association with relapse and OS, highlighting the importance of identifying clinically validated cut-offs for cfDNA analysis [38] [39] [40] and also underlining the limitations associated with analyzing a small patient cohort.

One main finding of our analysis was that cf*KRAS*<sup>mut</sup> positivity and CA19-9 elevation are only partially overlapping and that combining both parameters identifies a larger cohort of patient with poor outcome. Several studies have suggested integration of established and experimental protein biomarkers with cfDNA analysis for pancreatic cancer early diagnostics [27] [26] [41] [42] [43], identification of minimal residual disease [44] and molecular monitoring for advanced disease [40]. Our approach is focused on clinical applicability and feasibility through integration of the two relatively easy-to-assess biomarkers cf*KRAS*<sup>mut</sup> analysis and CA19-9. And while our data clearly show that CA19-9 and cf*KRAS*<sup>mut</sup> levels each have their own distinct advantages and disadvantages and that integrating them for analysis might be superior to analysing them individually, the question of how best to integrate both biomarkers for clinical practice remains challenging, which is also illustrated by the analysis of single patient's disease course in our cohort.

Finally, much larger trials will be required to study the potential on biomarker-based therapeutic intervention for pancreatic cancer. Systemic treatment options for PDAC are limited to a small number of

combination chemotherapy regimens (PRODIGE 11, NEJM 2011, MPCAT trials NEJM 2013) and some recent developments in personalized treatment based on molecular profiling (POLO trial NEJM 2019). A switch of adjuvant chemotherapy regimen based on molecular monitoring appears feasible yet need extensive clinical validation in interventional trials, especially since established adjuvant treatment standards took so many years to establish.

In summary, our study proposed a clinically feasible way to assay cf*KRAS*<sup>mut</sup> together with CA19-9 in patients following curative resection of PDAC. Through combination of both markers, patients could be better stratified in terms of relapse risk and overall prognosis.

## List Of Abbreviations

cfDNA	Cell-free DNA
cf <i>KRAS</i> <sup>mut</sup>	Mutated cell-free plasma <i>KRAS</i>
ctDNA	Tumor-derived cell-free nucleic acids
ddPCR	Droplet digital PCR
IDT	Integrated DNA Technologies
IRB	Institutional review board
HR	Hazard ratio
LOB	Limit of blank
LOD	Limit of detection
mFOLFIRINOX	5-fluorouracil, irinotecan, oxaliplatin, leucovorin
NGS	Next generation sequencing
OS	Overall survival
PCR	Polymerase chain reaction
PDAC	Pancreatic ductal adenocarcinoma
pts	Patients
RFS	Recurrence-free survival
SNV	Single nucleotide variants

## **Declarations**

### ***Ethics approval and consent to participate***

Local institutional review board (IRB) approved all study procedures (EK48/18). All patients provided written informed consent for sample collection and analysis.

### ***Availability of data and materials***

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### ***Competing interests***

The authors declare that they have no competing interests.

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### ***Authors' contributions***

SH collected, analyzed and interpreted the patient data. SH, DA and RF were major contributors in writing the manuscript. SH, MB, UW and RF conceptualized the study. RF supervised the data collection, analysis and writing of the manuscript. JH, MF, RK, UP, FS, NB and JD contributed to data collection and manuscript edition. All authors read and approved the final manuscript.

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## **References**

1. Rawla P, Sunkara T, Gaduputi V. Epidemiology of Pancreatic Cancer: Global Trends, Etiology and Risk Factors. *World journal of oncology*. 2019;10(1):10–27.
2. <https://seer.cancer.gov/statfacts/html/pancreas.html>. Accessed 14 August 2020.
3. Huang L, Jansen L, Balavarca Y, Babaei M, van der Geest L, Lemmens V, Van Eycken L, De Schutter H, Johannesen TB, Primic-Žakelj M, et al. Stratified survival of resected and overall pancreatic cancer patients in Europe and the USA in the early twenty-first century: a large, international population-based study. *BMC Med*. 2018;16(1):125–5.
4. Wittel UA, Lubgan D, Ghadimi M, Belyaev O, Uhl W, Bechstein WO, Grützmann R, Hohenberger WM, Schmid A, Jacobasch L, et al. Consensus in determining the resectability of locally progressed pancreatic ductal adenocarcinoma – results of the Conko-007 multicenter trial. *BMC Cancer*. 2019;19(1):979.
5. Chakraborty S, Singh S. Surgical resection improves survival in pancreatic cancer patients without vascular invasion- a population based study. *Ann Gastroenterol*. 2013;26(4):346–52.
6. Katz MH, Wang H, Fleming JB, Sun CC, Hwang RF, Wolff RA, Varadhachary G, Abbruzzese JL, Crane CH, Krishnan S, et al. Long-term survival after multidisciplinary management of resected pancreatic adenocarcinoma. *Ann Surg Oncol*. 2009;16(4):836–47.
7. Oettle H, Post S, Neuhaus P, Gellert K, Langrehr J, Ridwelski K, Schramm H, Fahlke J, Zuelke C, Burkart C, et al. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. *Jama*. 2007;297(3):267–77.
8. Smeenk HG, van Eijck CH, Hop WC, Erdmann J, Tran KC, Debois M, van Cutsem E, van Dekken H, Klinkenbijn JH, Jeekel J. Long-term survival and metastatic pattern of pancreatic and periampullary cancer after adjuvant chemoradiation or observation: long-term results of EORTC trial 40891. *Annals of surgery*. 2007;246(5):734–40.
9. Sperti C, Pasquali C, Piccoli A, Pedrazzoli S. Recurrence after resection for ductal adenocarcinoma of the pancreas. *World journal of surgery*. 1997;21(2):195–200.
10. Raut CP, Tseng JF, Sun CC, Wang H, Wolff RA, Crane CH, Hwang R, Vauthey J-N, Abdalla EK, Lee JE, et al. Impact of resection status on pattern of failure and survival after pancreaticoduodenectomy for pancreatic adenocarcinoma. *Annals of surgery*. 2007;246(1):52–60.
11. Conroy T, Hammel P, Hebbar M, Ben Abdelghani M, Wei AC, Raoul J-L, Choné L, Francois E, Artru P, Biagi JJ, et al: **FOLFIRINOX or Gemcitabine as Adjuvant Therapy for Pancreatic Cancer**. 2018, 379(25):2395–2406.
12. Ying H, Dey P, Yao W, Kimmelman AC, Draetta GF, Maitra A, DePinho RA. Genetics and biology of pancreatic ductal adenocarcinoma. *Genes Dev*. 2016;30(4):355–85.
13. Yao W, Maitra A, Ying H. **Recent insights into the biology of pancreatic cancer**. *EBioMedicine* 2020, 53.
14. Golan T, Hammel P, Reni M, Van Cutsem E, Macarulla T, Hall MJ, Park JO, Hochhauser D, Arnold D, Oh DY, et al. Maintenance Olaparib for Germline BRCA-Mutated Metastatic Pancreatic Cancer. *N Engl J*

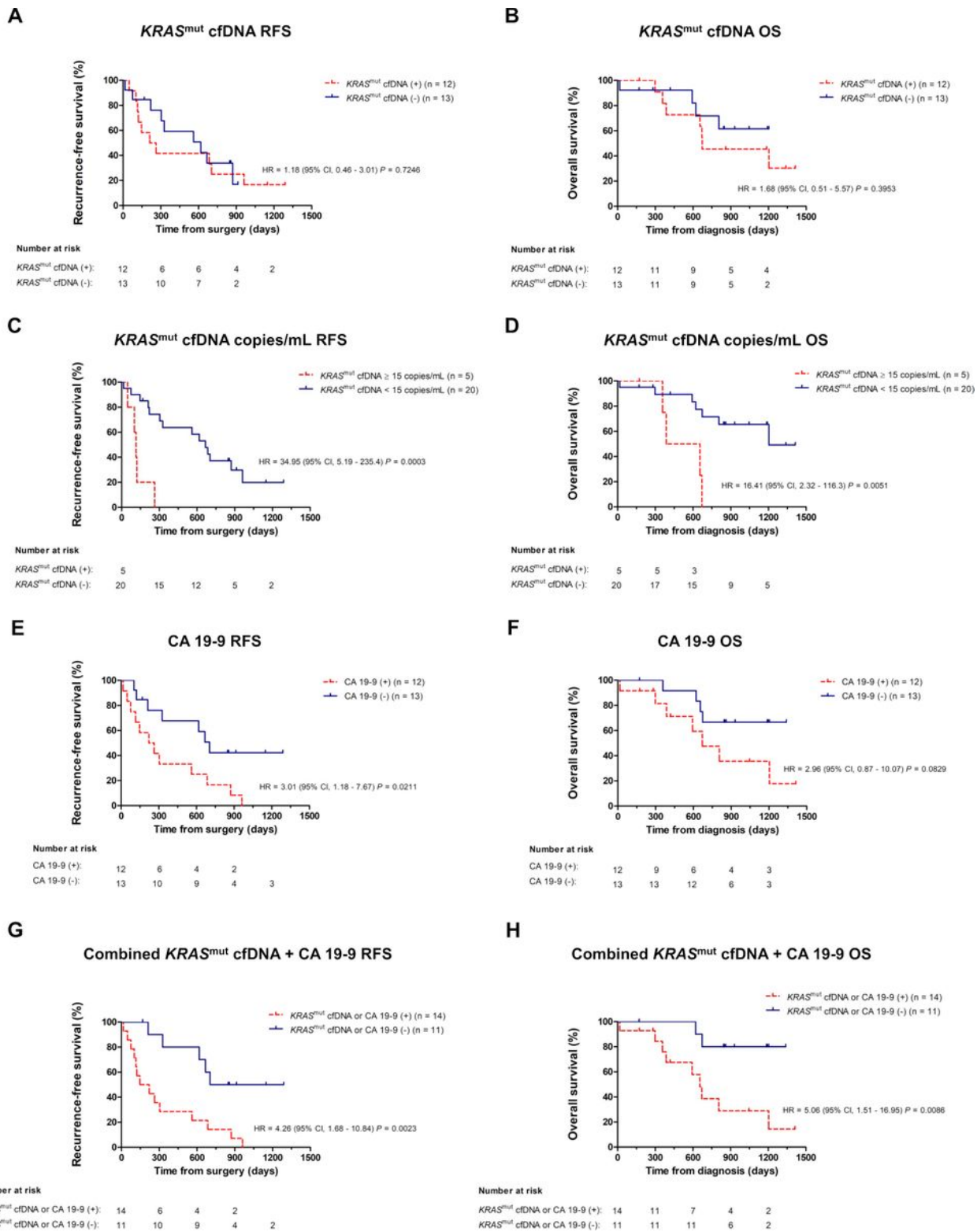
- Med. 2019;381(4):317–27.
15. Grinshpun A, Zerbiv Y, Roszik J, Subbiah V, Hubert A. Beyond KRAS: Practical Molecular Targets in Pancreatic Adenocarcinoma. *Case Reports in Oncology*. 2019;12(1):7–13.
  16. Aguirre AJ. **Oncogenic NRG1 Fusions: A New Hope for Targeted Therapy in Pancreatic Cancer**. 2019, 25(15):4589–4591.
  17. Khater F, Langlois S, Cassart P, Roy AM, Lajoie M, Healy J, Richer C, St-Onge P, Piché N, Perreault S, et al. Recurrent somatic BRAF insertion (p.V504\_R506dup): a tumor marker and a potential therapeutic target in pilocytic astrocytoma. *Oncogene*. 2019;38(16):2994–3002.
  18. Amin S, Baine M, Meza J, Lin C. The impact of neoadjuvant and adjuvant immunotherapy on the survival of pancreatic cancer patients: a retrospective analysis. *BMC Cancer*. 2020;20(1):538.
  19. Alix-Panabières C, Pantel K. Clinical Applications of Circulating Tumor Cells and Circulating Tumor DNA as Liquid Biopsy. *Cancer discovery*. 2016;6(5):479–91.
  20. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, Bartlett BR, Wang H, Luber B, Alani RM, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Science translational medicine*. 2014;6(224):224ra224.
  21. Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2014;32(6):579–86.
  22. Macías M, Alegre E, Díaz-Lagares A, Patiño A, Pérez-Gracia JL, Sanmamed M, López-López R, Varo N, González A. Liquid Biopsy: From Basic Research to Clinical Practice. *Adv Clin Chem*. 2018;83:73–119.
  23. Gormally E, Caboux E, Vineis P, Hainaut P. Circulating free DNA in plasma or serum as biomarker of carcinogenesis: practical aspects and biological significance. *Mutat Res*. 2007;635(2–3):105–17.
  24. Kato S, Janku F. Cell-free DNA as a novel marker in cancer therapy. *Biomark Med*. 2015;9(7):703–12.
  25. Meng P, Wei J, Geng Y, Chen S, Terpstra MM, Huang Q, Zhang Q, Su Z, Yu W, Su M, et al. Targeted sequencing of circulating cell-free DNA in stage II-III resectable oesophageal squamous cell carcinoma patients. *BMC Cancer*. 2019;19(1):818.
  26. Cohen JD, Javed AA, Thoburn C, Wong F, Tie J, Gibbs P, Schmidt CM, Yip-Schneider MT, Allen PJ, Schattner M, et al: **Combined circulating tumor DNA and protein biomarker-based liquid biopsy for the earlier detection of pancreatic cancers**. 2017, 114(38):10202–10207.
  27. Cohen JD, Li L, Wang Y, Thoburn C, Afsari B, Danilova L, Douville C, Javed AA, Wong F, Mattox A, et al: **Detection and localization of surgically resectable cancers with a multi-analyte blood test**. 2018, 359(6378):926–930.
  28. Zill OA, Greene C, Sebisano D, Siew LM, Leng J, Vu M, Hendifar AE, Wang Z, Atreya CE, Kelley RK, et al. Cell-Free DNA Next-Generation Sequencing in Pancreatobiliary Carcinomas. *Cancer discovery*. 2015;5(10):1040–8.
  29. Hussung S, Follo M, Klar RFU, Michalczyk S, Fritsch K, Nollmann F, Hipp J, Duyster J, Scherer F, von Bubnoff N, et al. Development and Clinical Validation of Discriminatory Multitarget Digital Droplet

- PCR Assays for the Detection of Hot Spot KRAS and NRAS Mutations in Cell-Free DNA. *The Journal of molecular diagnostics: JMD*. 2020;22(7):943–56.
30. Bernard V, Kim DU, San Lucas FA, Castillo J, Allenson K, Mulu FC, Stephens BM, Huang J, Semaan A, Guerrero PA, et al. Circulating Nucleic Acids Are Associated With Outcomes of Patients With Pancreatic Cancer. *Gastroenterology*. 2019;156(1):108–18.e104.
  31. Watanabe F, Suzuki K, Tamaki S, Abe I, Endo Y, Takayama Y, Ishikawa H, Kakizawa N, Saito M, Futsuhara K, et al. Longitudinal monitoring of KRAS-mutated circulating tumor DNA enables the prediction of prognosis and therapeutic responses in patients with pancreatic cancer. *PLoS One*. 2019;14(12):e0227366–6.
  32. Sausen M, Phallen J, Adleff V, Jones S, Leary RJ, Barrett MT, Anagnostou V, Parpart-Li S, Murphy D, Kay Li Q, et al. Clinical implications of genomic alterations in the tumour and circulation of pancreatic cancer patients. *Nat Commun*. 2015;6:7686–6.
  33. Cheng H, Liu C, Jiang J, Luo G, Lu Y, Jin K, Guo M, Zhang Z, Xu J, Liu L, et al. Analysis of ctDNA to predict prognosis and monitor treatment responses in metastatic pancreatic cancer patients. *International journal of cancer*. 2017;140(10):2344–50.
  34. Patel H, Okamura R, Fanta P, Patel C, Lanman RB, Raymond VM, Kato S, Kurzrock R. Clinical correlates of blood-derived circulating tumor DNA in pancreatic cancer. *J Hematol Oncol*. 2019;12(1):130–0.
  35. Pietrasz D, Pécuchet N, Garlan F, Didelot A, Dubreuil O, Doat S, Imbert-Bismut F, Karoui M, Vaillant JC, Taly V, et al. Plasma Circulating Tumor DNA in Pancreatic Cancer Patients Is a Prognostic Marker. *Clinical cancer research: an official journal of the American Association for Cancer Research*. 2017;23(1):116–23.
  36. Gall TMH, Belete S, Khanderia E, Frampton AE, Jiao LR. Circulating Tumor Cells and Cell-Free DNA in Pancreatic Ductal Adenocarcinoma. *Am J Pathol*. 2019;189(1):71–81.
  37. Malapelle U, Mayo de-Las-Casas C, Rocco D, Garzon M, Pisapia P, Jordana-Ariza N, Russo M, Sgariglia R, De Luca C, Pepe F, et al. Development of a gene panel for next-generation sequencing of clinically relevant mutations in cell-free DNA from cancer patients. *Br J Cancer*. 2017;116(6):802–10.
  38. Crowley E, Di Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. *Nature Reviews Clinical Oncology*. 2013;10(8):472–84.
  39. Fleischhacker M, Schmidt B. Circulating nucleic acids (CNAs) and cancer—a survey. *Biochim Biophys Acta*. 2007;1775(1):181–232.
  40. Bronkhorst AJ, Ungerer V, Holdenrieder S. The emerging role of cell-free DNA as a molecular marker for cancer management. *Biomol Detect Quantif*. 2019;17:100087–7.
  41. Nazli O, Bozdog AD, Tansug T, Kir R, Kaymak E. The diagnostic importance of CEA and CA 19 – 9 for the early diagnosis of pancreatic carcinoma. *Hepato-gastroenterology*. 2000;47(36):1750–2.
  42. Capello M, Bantis LE, Scelo G, Zhao Y, Li P, Dhillon DS, Patel NJ, Kundnani DL, Wang H, Abbruzzese JL, et al: **Sequential Validation of Blood-Based Protein Biomarker Candidates for Early-Stage Pancreatic Cancer**. *JNCI: Journal of the National Cancer Institute* 2016, 109(4).

43. Herreros-Villanueva M, Bujanda L. Non-invasive biomarkers in pancreatic cancer diagnosis: what we need versus what we have. *Ann Transl Med.* 2016;4(7):134–4.
44. Chae YK, Oh MS. Detection of Minimal Residual Disease Using ctDNA in Lung Cancer: Current Evidence and Future Directions. *Journal of thoracic oncology: official publication of the International Association for the Study of Lung Cancer.* 2019;14(1):16–24.

## Figures

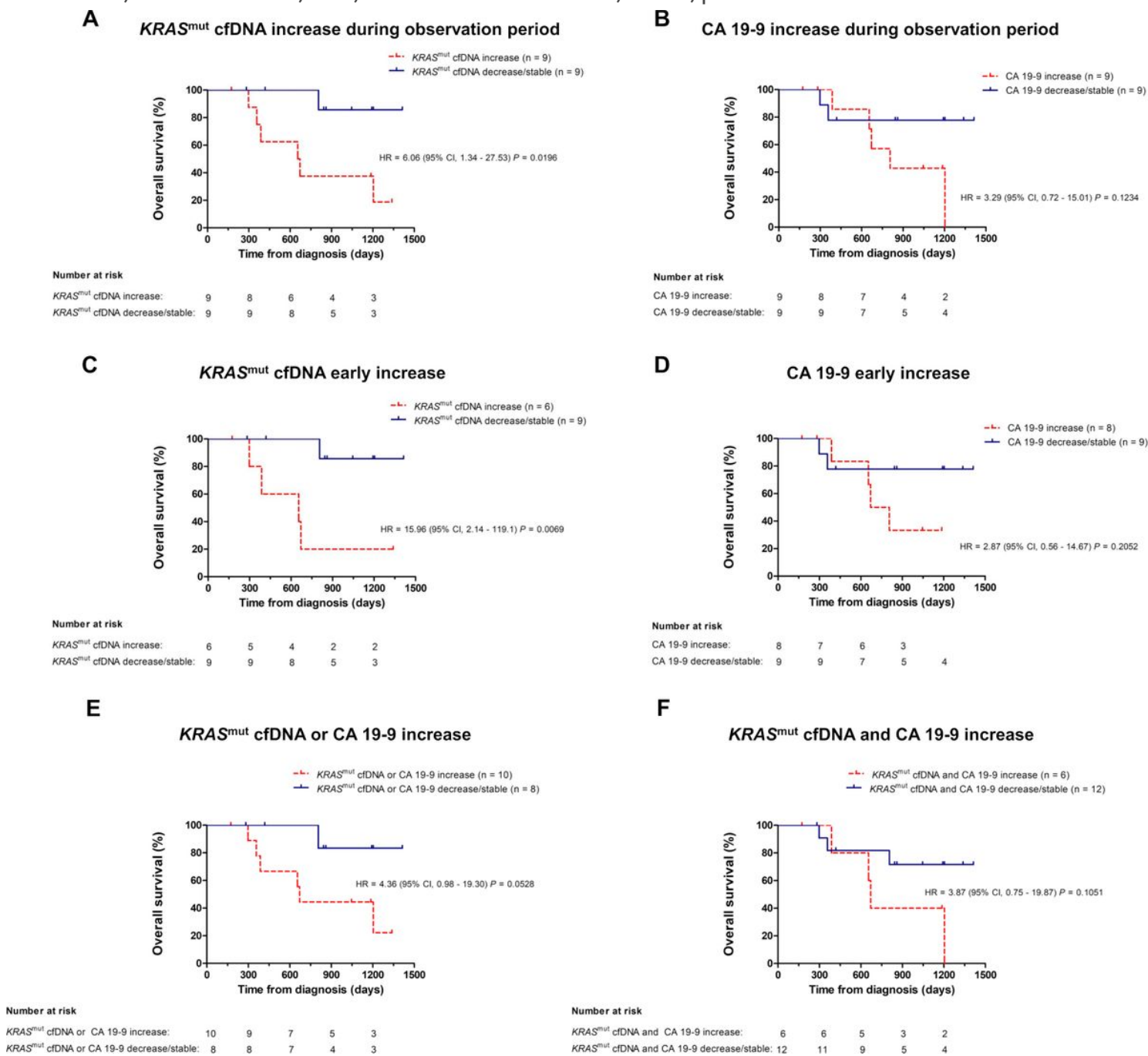




**Figure 1**

Association of cfKRAS<sup>mut</sup> detection and elevated CA19-9 levels with survival endpoints (A, B) Kaplan-Meier estimates of RFS (A) and OS (B) for patients following curative resection of PDAC with versus without detectable cfKRAS<sup>mut</sup> at any time point during study period. (C, D). A more stringent cfKRAS<sup>mut</sup> cut-off level of > 15 copies/mL plasma was chosen. (E, F) Kaplan-Meier estimates of RFS (E) and OS (F) for resected PDAC patients with elevated (> 36 U/mL) versus normal (≤ 36 U/mL) CA19-9 levels at any

time point during observation period. (G, H) Kaplan-Meier estimates of RFS (G) and OS (H) for resected PDAC patients with either CA19-9 positivity or cfKRASmut levels > 15 copies/mL cfKRAS during study course. OS, overall survival; RFS, recurrence-free survival; PDAC, pancreatic ductal adenocarcinoma

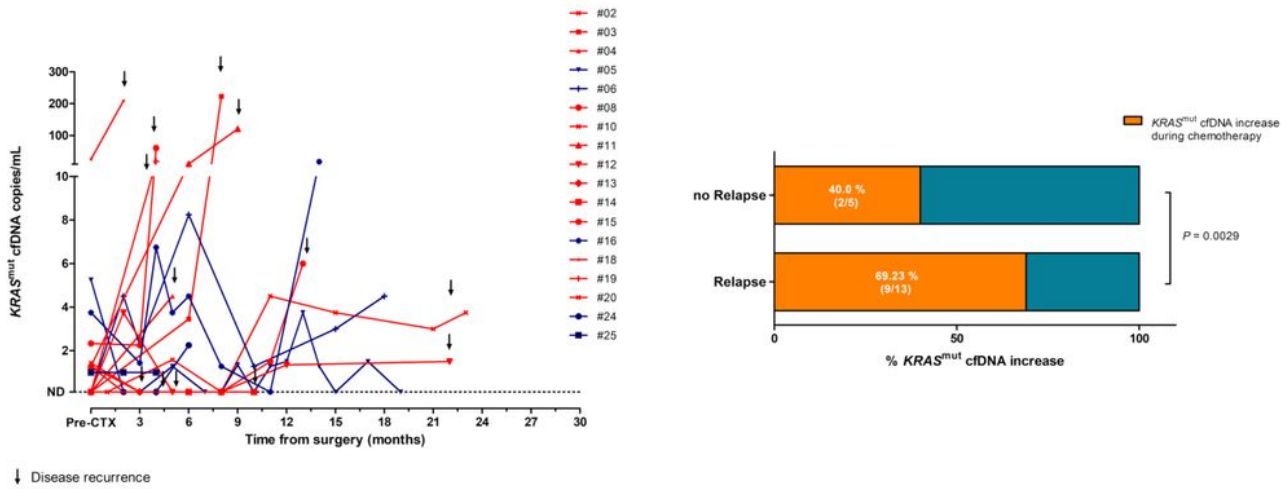


**Figure 2**

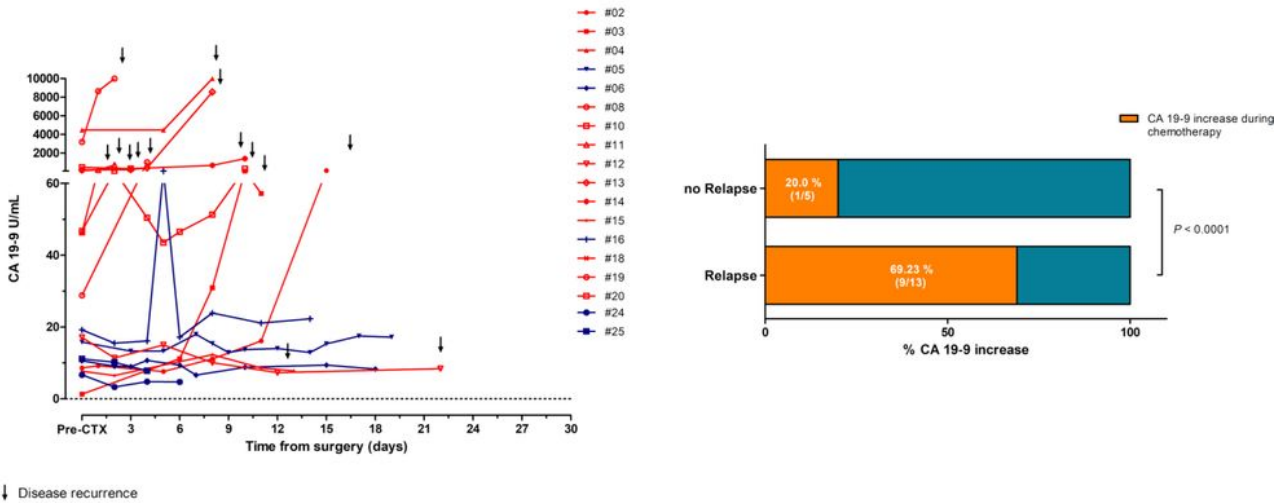
Association of cfKRASmut and CA19-9 dynamic changes with survival endpoints (A, B) Kaplan-Meier estimates of OS for resected PDAC patients with increase of cfKRASmut (A) or CA 19-9 (B) during observation period. (C, D) Kaplan-Meier estimates of OS for resected PDAC patients with early increase of cfKRASmut (A) or CA 19-9 (B) during observation period. Early increase was defined as increase within 6 months after surgery. (E) Kaplan-Meier estimates of OS for resected PDAC patients with combined early increase of cfKRASmut or CA 19-9. (F) Kaplan-Meier estimates of OS for resected PDAC patients with

combined early increase of cfKRASmut and CA 19-9. OS, overall survival; RFS, recurrence-free survival; PDAC, pancreatic ductal adenocarcinoma

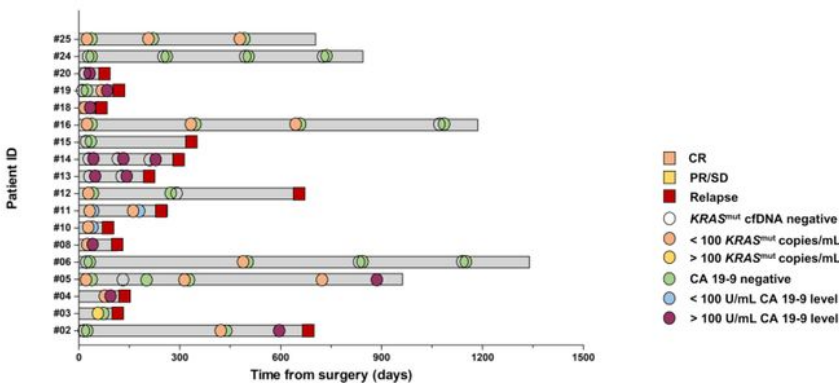
**A KRAS<sup>mut</sup> cfDNA kinetics during observation period**



**CA 19-9 kinetics during observation period**



**B**



**Figure 3**

Longitudinal cfKRASmut and CA 19-9 monitoring (A) Top left: Absolute levels of cfKRASmut during observation period. Patients with relapse during study period are marked red. Black arrow mark the time of disease recurrence. Top right: Relapse versus non-relapse patients with increase in cfKRASmut during

observation period. Fisher's exact test was used to test for statistical significance between the two groups. P values < 0.05 were considered significant. Bottom left: Absolute levels of CA 19-9 during observation period. Patients with relapse are marked red. Black arrows mark the time of disease recurrence. Bottom right: Relapse versus non-relapse patients with increase in cfKRASmut during observation period. Fisher's exact test was used to test for statistical significance between the two groups. P values < 0.05 were considered significant. (B) Swimmers plot of disease course of resected PDAC patients. cfKRASmut and CA 19-9 analysis in blood were compared to clinical course of disease before and during adjuvant chemotherapy. CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

## Supplementary Files

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