

# Detection and quantitation of ctDNA *KRAS* mutations from patients with unresectable pancreatic cancer

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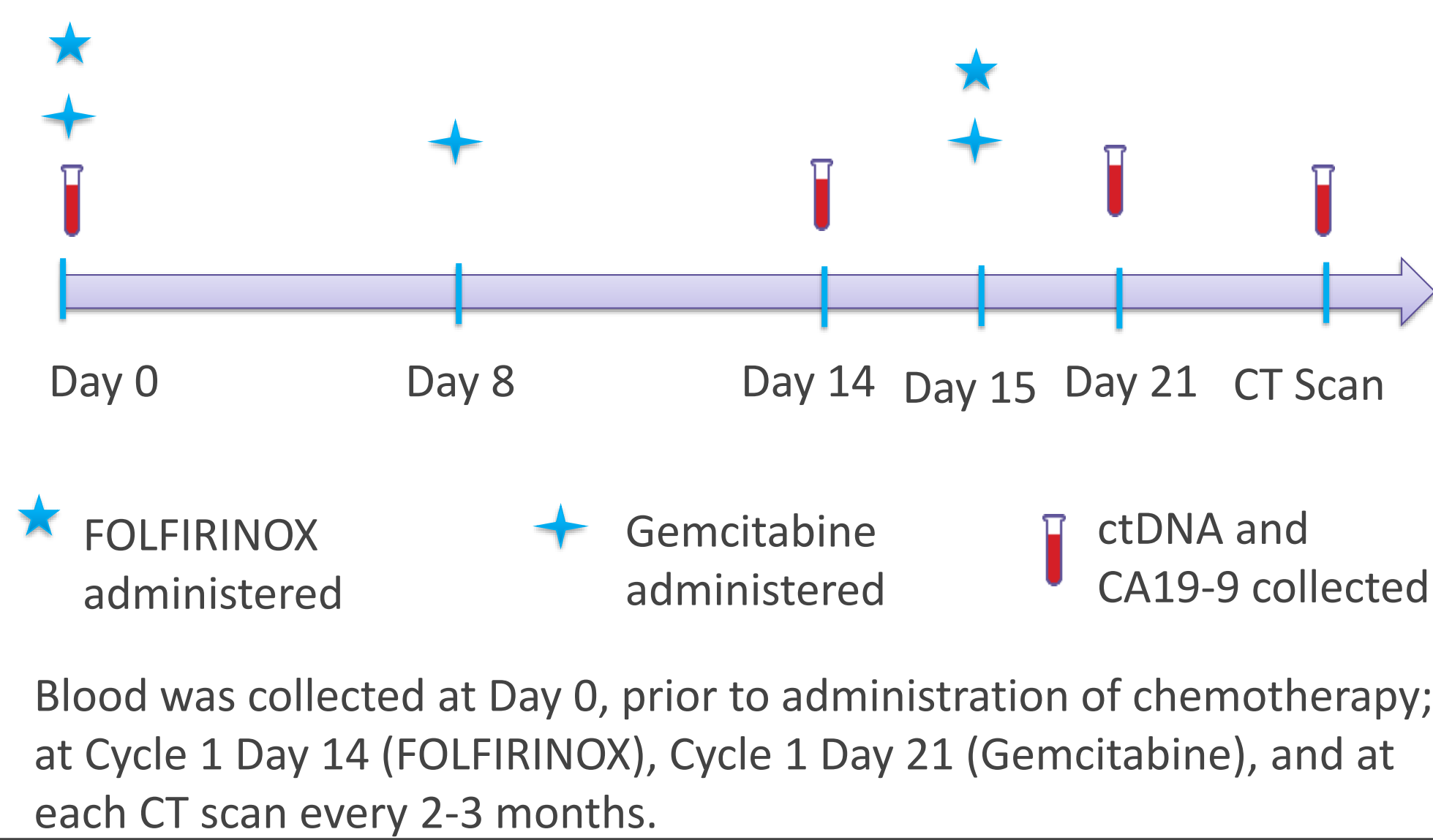
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## Introduction

- A diagnostic and prognostic clinical tool with very high analytical sensitivity, clinical sensitivity, and quantitation is needed for therapeutic response monitoring.
- An estimated 90% of pancreatic cancers harbor somatic *KRAS* G12/G13 mutations.
- Recent studies on small numbers of patients demonstrate widely variable *KRAS* ctDNA sensitivity (27 – 71%)<sup>1</sup>.
- Accurate identification and quantitation of ctDNA *KRAS* mutation copies would be an improved prognostic and therapeutic response biomarker over CA19-9 which is known to be uninformative in 5-10% of patients with pancreatic cancer.

## Study Design and Objectives

Figure 1: Blood collection timepoints for 210 patients with unresectable pancreatic cancer



### Primary Objective:

- Examine *KRAS* G12/13 detection rate in plasma of patients with unresectable, locally advanced or metastatic pancreatic cancer.

### Secondary Objectives:

- Examine association between baseline *KRAS* levels in plasma and patient outcomes.
- Examine correlation between changes in *KRAS* levels in plasma and changes in tumor size by radiographic assessment following treatment with chemotherapy.

- Pretreatment (baseline) and longitudinal blood samples (1 - 4 mL) were prospectively collected from patients with unresectable pancreatic cancer through the Danish BIOPAC study (Figure 1; Table 1).
- ctDNA *KRAS* G12A/C/D/R/S/V, and G13D mutations were PCR enriched, sequenced by next generation sequencing (NGS), quantitated, and standardized.
- Standard curves were generated from a sample set with known numbers of spike-in copies for mutant *KRAS* molecules which were assayed in parallel with patient samples starting with PCR enrichment of mutant *KRAS* DNA followed by NGS.
- The number of mutant copies detected was standardized by normalizing the number of copies detected in the sample to a constant number of calculated genome equivalents (GEq) of wild type DNA across all samples evaluated.

## Patient Cohort

- 210 of 236 patients had evaluable baseline samples (Table 1; Figure 2).

Figure 2: Patient Baseline Sample Consort

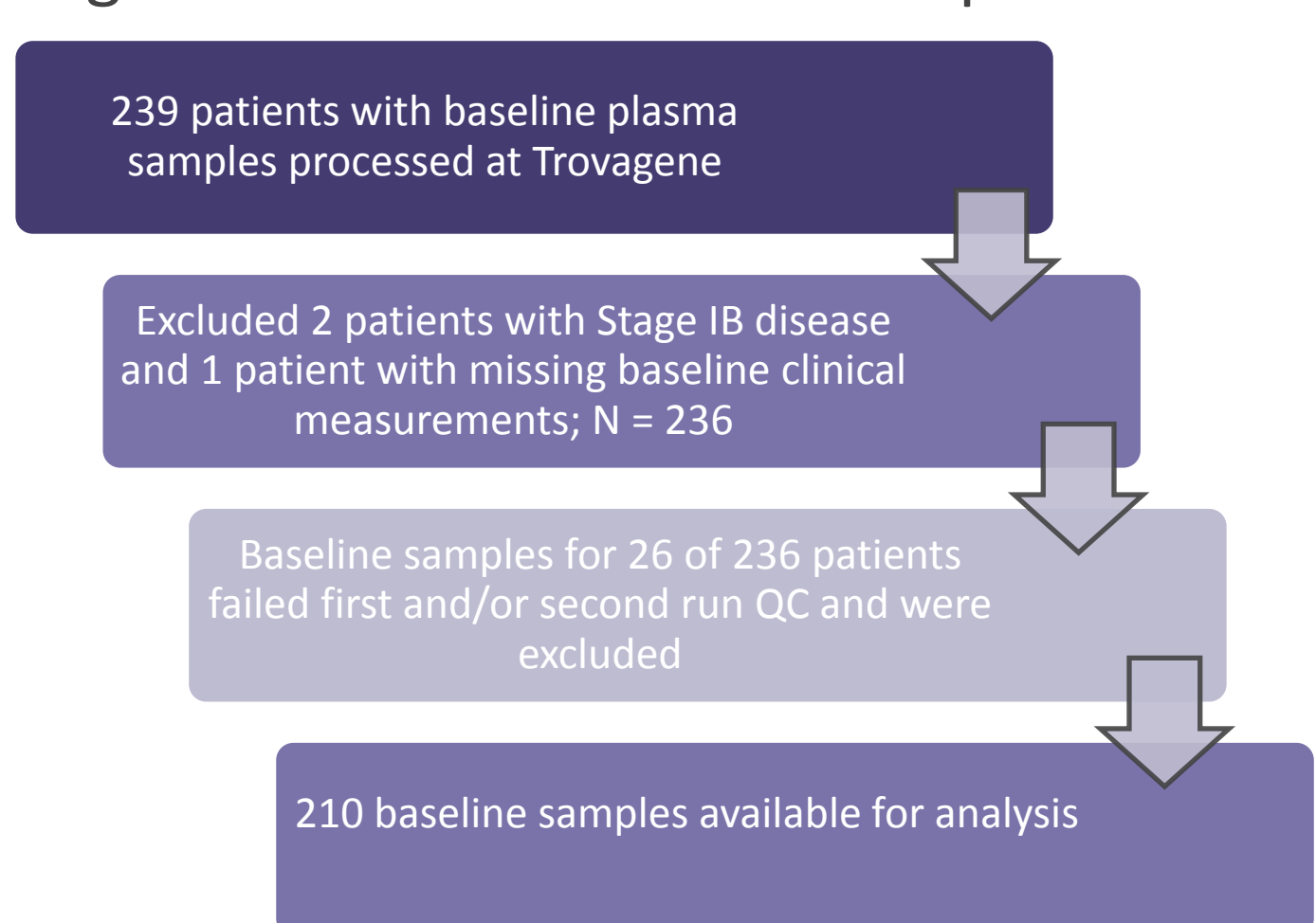


Table 1: Characteristics of the 210 patients with evaluable baseline plasma samples

		Number of Patients	Percentage of Patients
Gender	Male	107	51.0
	Female	103	49.0
Median Age	67.5 years		Range 42 – 89 years
Cancer Stage	III	43	20.5
	IV	167	79.5
Presentation	Locally Advanced	43	20.5
	Metastatic	167	79.5
Therapy	Gemcitabine	139	66.2
	FOLFIRINOX	71	33.8

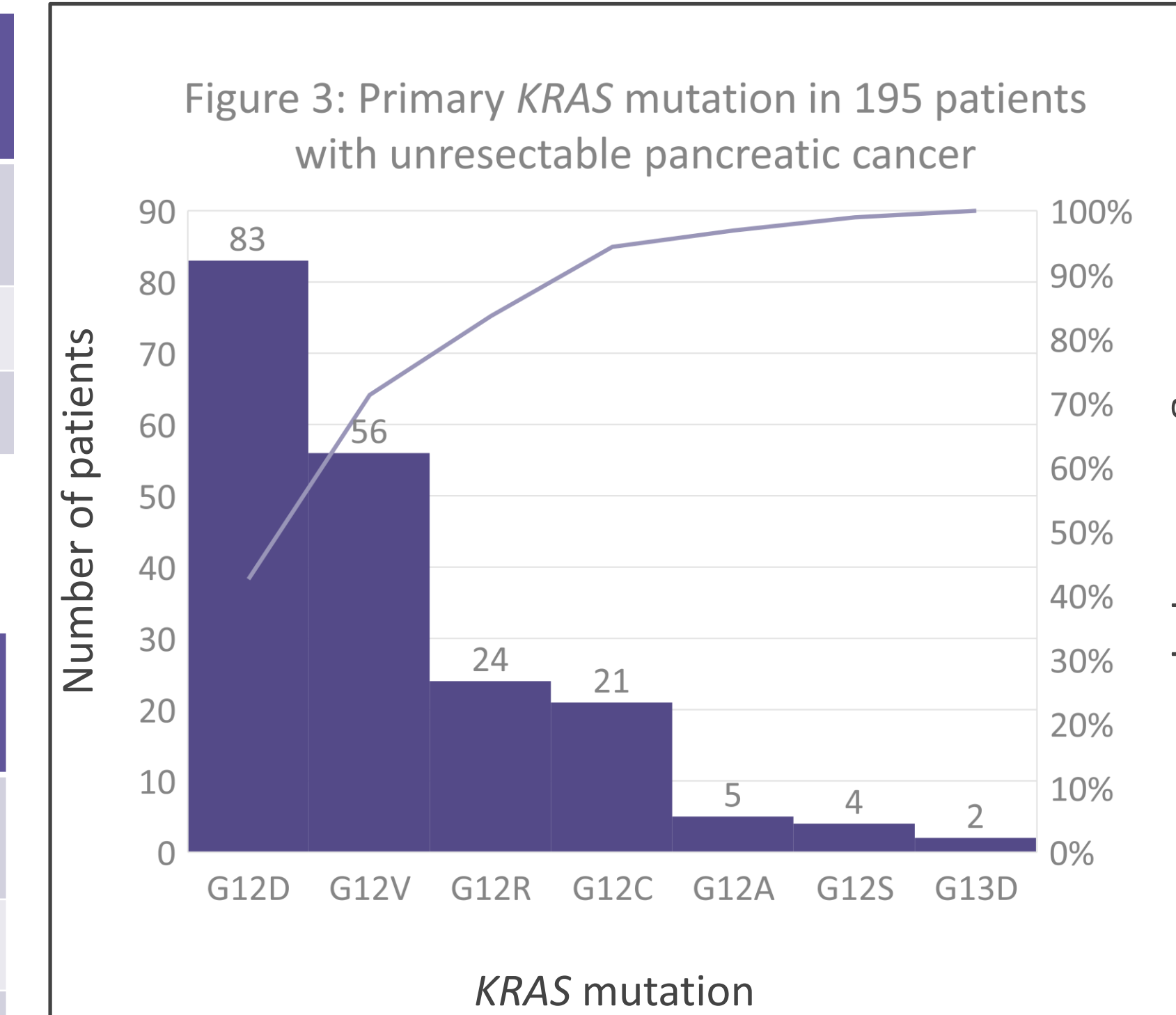
- ctDNA *KRAS* mutation detection rate at baseline was 92.9% (95% CI 88.5% to 95.9%) with a median of 280.6 copies per 100,000 (10<sup>5</sup>) GEq (Table 2).
- The majority of patients (66%) had somatic *KRAS* G12D and G12V mutations (Figure 3).
- Baseline CA19-9 was available for 155 patients and ranged from 3 – 608,500 U/mL (median 1000 U/mL). Eighteen patients (11.6%) had CA19-9 below threshold (<37 U/mL) (Table 3; Figure 4).

Table 2: *KRAS* status by cancer presentation. *KRAS* was detected in 95.8% of patients with metastatic disease versus 81.4% in patients with locally advanced disease.

Presentation	<i>KRAS</i> not detected	<i>KRAS</i> detected	Total
Locally Advanced	8	35	43
Metastatic	7	160	167
Total	15	195	210

Table 3: CA19-9 by cancer presentation. Consistent with previously published data, CA19-9 was uninformative in 11.6% of patients (18/155).

Presentation	CA 19-9 < 37	CA 19-9 ≥ 37	CA 19-9 not available	Total
Locally Advanced	3	29	11	43
Metastatic	15	108	44	167
Total	18	137	55	210



- Patients presenting with metastatic disease had a significantly higher median number of *KRAS* mutations (458.8 versus 56.1 copies per 10<sup>5</sup> GEq; Wilcoxon's p-value < 0.0001) which represents an approximately 8.2 fold difference.
- Patients presenting with metastatic disease had significantly higher CA19-9 (1637 versus 465 U/mL; Wilcoxon's p-value < 0.0073) at baseline than those with locally advanced cancer which represents a 3.9 fold difference.

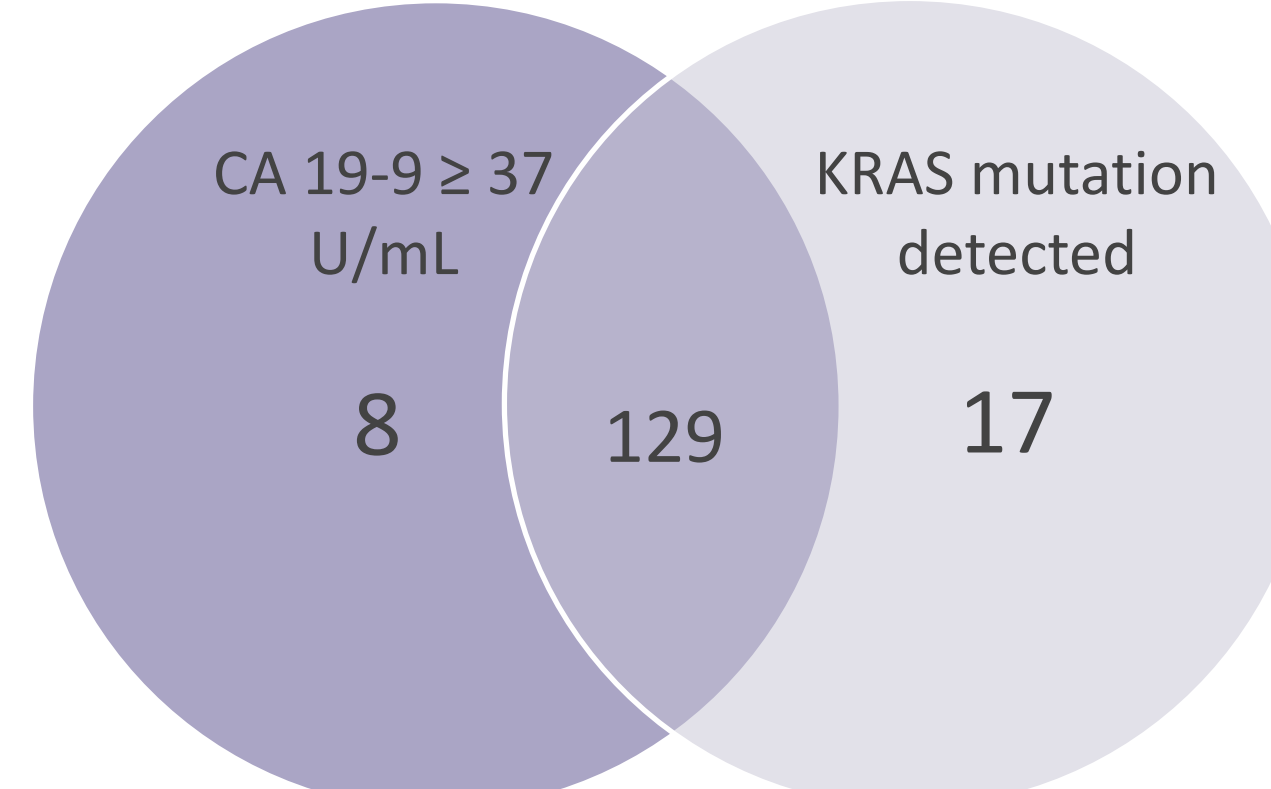


Figure 4: Concordance between *KRAS* mutation status and CA19-9 in 155 patients with baseline CA19-9 available. Twenty-five patients (16.2%) had discordant ctDNA *KRAS* and CA19-9 results. Of these 25, ctDNA *KRAS* identified 52% more patients than CA19-9. One patient had undetectable ctDNA *KRAS* and low CA19-9 (<37 U/mL). 55 patients did not have CA19-9 available at baseline. 49 (89%) were positive for *KRAS*.

- Patients who had high *KRAS* (above the median) appear to have a worse overall survival than those with low *KRAS* (Table 4; Figure 5).

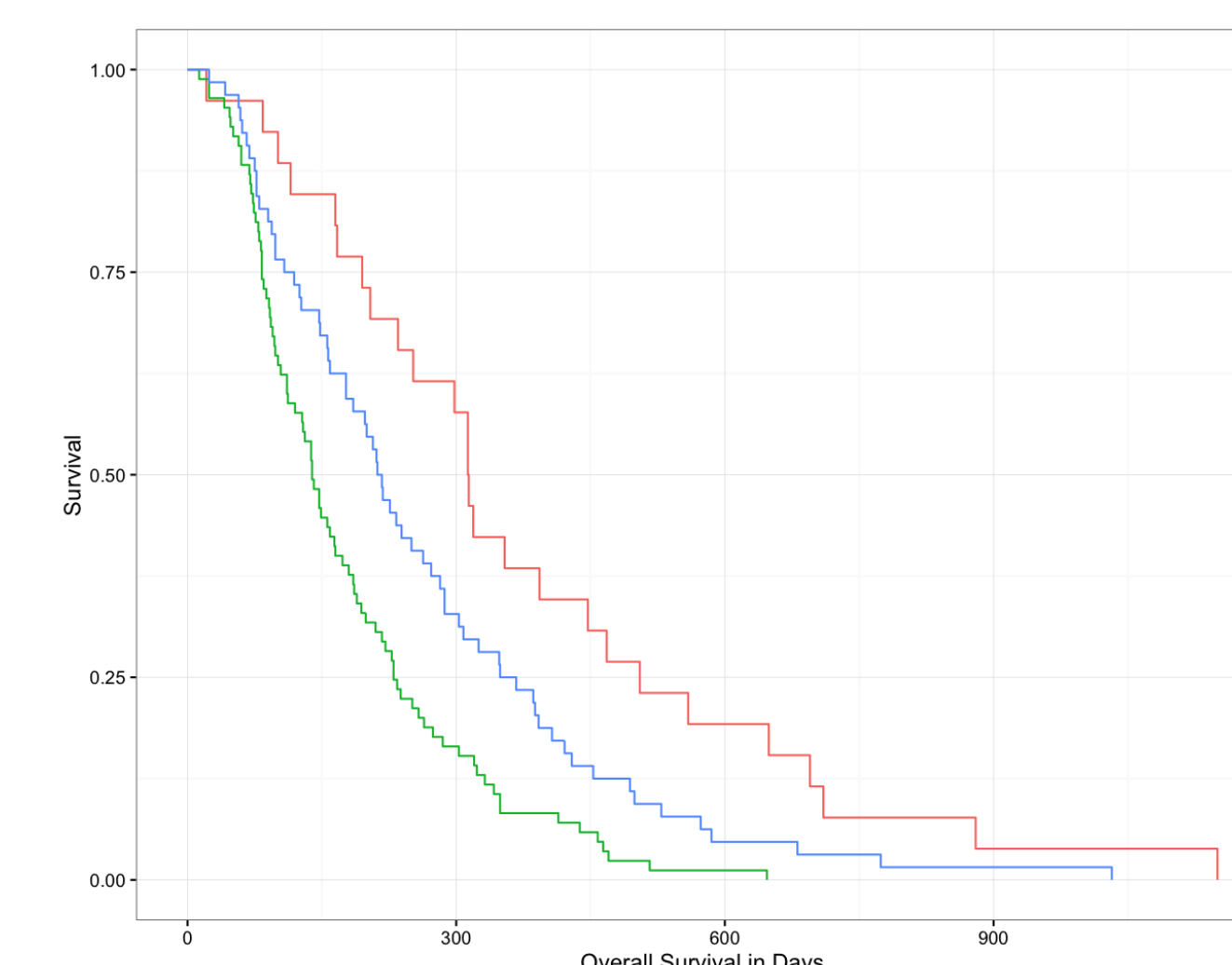
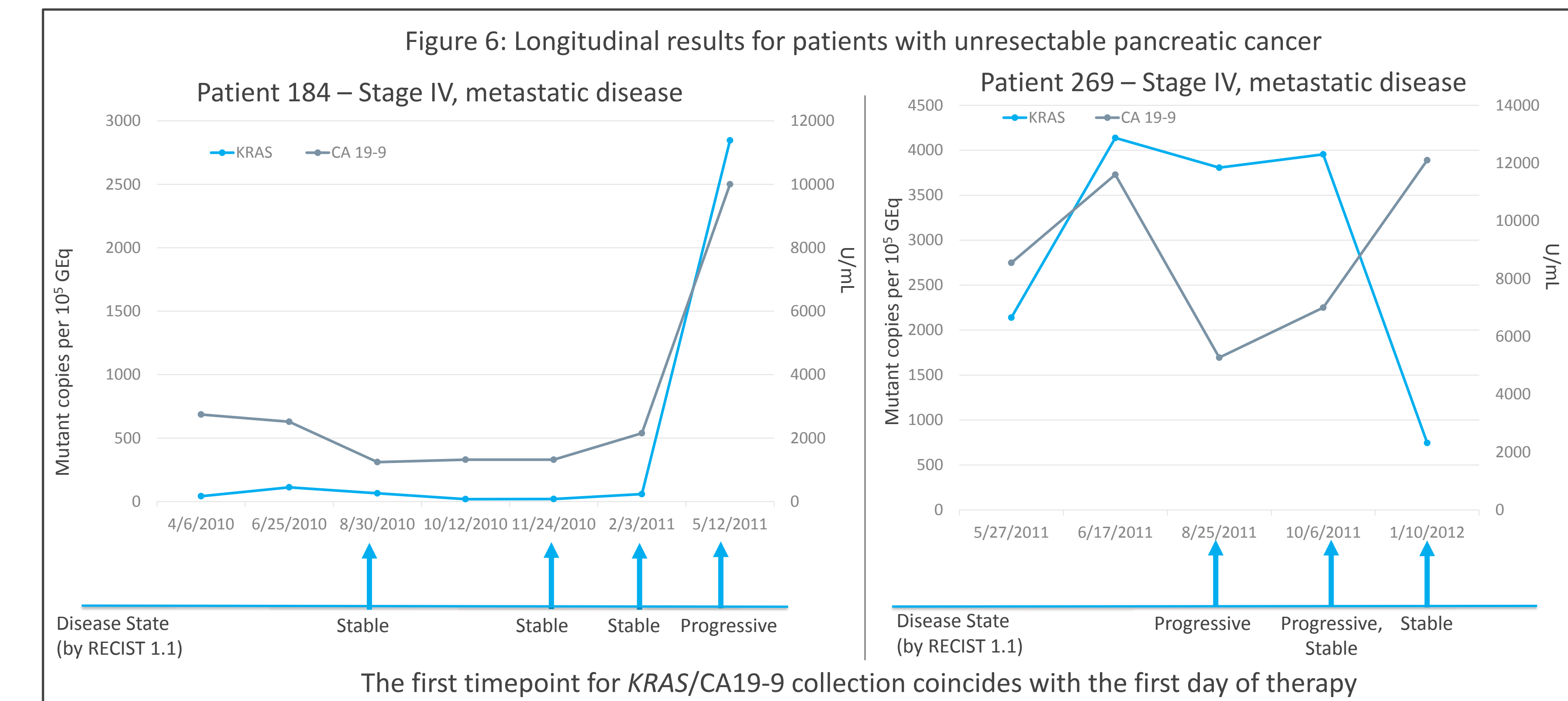


Table 4: Figure 5: Kaplan Meier demonstrating overall survival by presentation and *KRAS* status (above or below the median) in patients with detected *KRAS*. Fourteen patients are alive.

Presentation / <i>KRAS</i> status	Number of Patients	Median Overall Survival (Days)	95% confidence Interval
Locally Advanced, Low <i>KRAS</i>	26	314	235 - 505
Locally advanced, High <i>KRAS</i> *	5	-	-
Metastatic, Low <i>KRAS</i>	64	214	177 - 282
Metastatic, High <i>KRAS</i>	85	139	112 - 180

## Results

- Longitudinal timepoints demonstrate *KRAS* mutation corresponds with response.
- In one patient (184), increase in *KRAS* mutation copy load and CA19-9 correlated with progressive disease on imaging (Figure 6).
- In one patient (269), increase and decrease in *KRAS* mutation load corresponded with progressive and stable disease on imaging. *KRAS* mutation load was better correlated with disease than CA19-9 (Figure 6).



## Conclusions

- This is the largest, prospective dataset exploring ctDNA *KRAS* in unresectable pancreatic cancer.
- 92.9% of 210 patients with unresectable pancreatic cancer were positive for ctDNA *KRAS*.
  - This detection rate closely matches the published prevalence of *KRAS* in pancreatic cancer (90%), and outperforms previous studies, demonstrating the superior assay sensitivity.
  - ctDNA analysis offers a viable tissue biopsy alternative for determining *KRAS* mutation status, especially in late stage patients.
- ctDNA *KRAS* analysis identified 52% more patients as positive than CA19-9, demonstrating *KRAS* as an improved diagnostic tool.
- Individuals with metastatic disease had a 8.2 fold difference in median ctDNA *KRAS* mutation load at baseline versus a 3.9 fold difference in baseline CA19-9. *KRAS* may represent an improved biomarker for metastatic disease over CA19-9.
- In two representative patients, dynamic changes in *KRAS* mutation load were consistent with response by imaging and predicted progressive disease months in advance of progression by imaging.
- Quantitation of *KRAS* mutant copy load may provide a more informative biomarker for prognosis and monitoring for therapeutic response.