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

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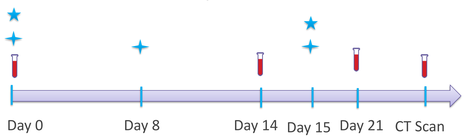
## Introduction

- 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%)[1].
- 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




Blood was collected at Day 0, prior to administration of chemotherapy; at Cycle 1 Day 14 (FOLFIRINOX), Cycle 1 Day 21 (Gemcitabine), and at each CT scan every 2-3 months.

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 (GEqs) 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

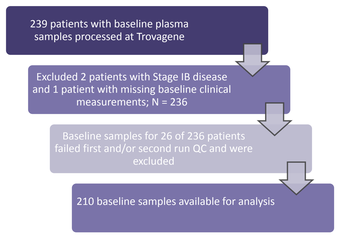



Table 1: Characteristics of the 210 patients with evaluable baseline plasma samples

| | | 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 |

## Results

- 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 ($10^5$) GEqs (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).

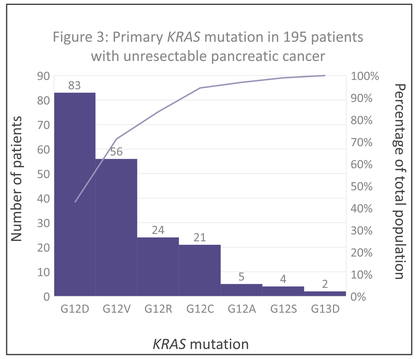
Table 2: *KRAS* status by cancer presentation. *KRAS* was detected in 95.8% of patients with metastatic disease versus 81.4% in patients with locally advanced disease.

| Presentation | KRAS not detected | KRAS 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 |

Figure 3: Primary *KRAS* mutation in 195 patients with unresectable pancreatic cancer




- Patients presenting with metastatic disease had a significantly higher median number of *KRAS* mutations (458.8 versus 56.1 copies per $10^5$ GEqs; 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.

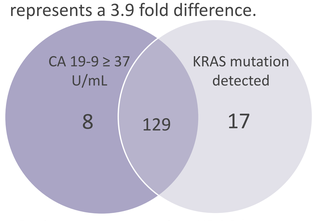



Figure 4: Concordance between *KRAS* mutation status and CA19-9 in 155 patients with baseline CA19-9 available. Twenty – five patients (16.2%) had discordant ctDNA *KRAS* and CA19-9 results. Of these 25, ctDNA *KRAS* identified 52% more patients than CA19-9. One patient had undetectable ctDNA *KRAS* and low CA19 (<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).

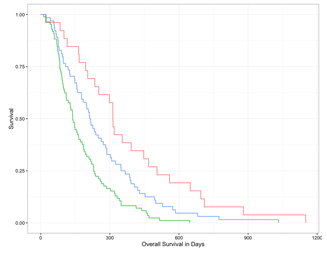


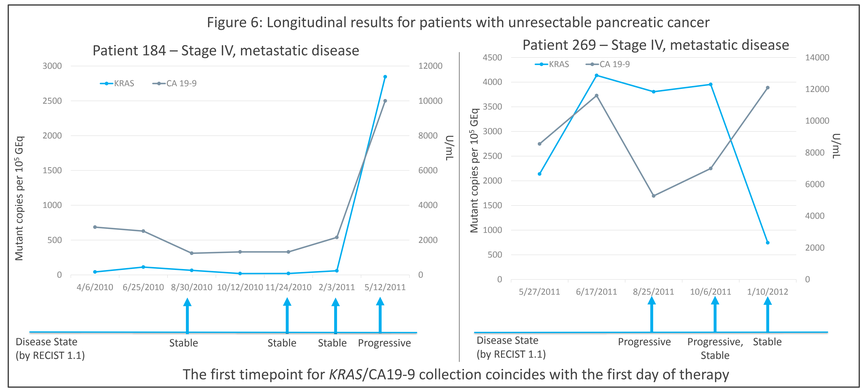

Table 4; Figure 5; Kaplan Meier demonstrating overall survival by presentation and *KRAS* status (above or below the median) in patients with detected *KRAS*. Fourteen patients are alive.

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

*Kaplan Meier curve not generated due to low n

- 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).

Figure 6: Longitudinal results for patients with unresectable pancreatic cancer




The first timepoint for *KRAS*/CA19-9 collection coincides with the first day of therapy

## 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 out performs 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.

References:
1. Castells, et al JCO. 1999; Tjensvoll, et al. Molec Oncol. 2015; Maire, et al. BJC 2002; Kinguasa, et al. Cancer 2015; Chen et al EJSO. 2010.

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