Comparative Levels of *KRAS* Mutations in Circulating Tumor DNA for Association with Overall Survival in Patients with Non-Resectable Pancreatic Cancer

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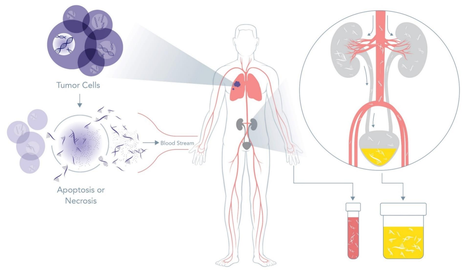
Abstract #: 5240

Background

Patients with non-resectable locally advanced or metastatic pancreatic cancer have a wide range of median time for overall survival (OS). Currently, there is a lack of diagnostic tools to predict patient outcome at diagnosis.

KRAS mutations are present in the vast majority of pancreatic tumors. The study objective was to determine if quantitative baseline and longitudinal monitoring of *KRAS* mutations from plasma circulating tumor DNA (ctDNA) could be used to stratify patients for predicting outcome.



Liquid biopsy: plasma and urine as sources of ctDNA

Clinical Study Design

- Archived plasma samples (up to 6 years) were prospectively collected from 182 patients (85 females and 97 males, median age 68, range 45-89 years) with locally advanced or metastatic pancreatic cancer included in the Danish BIOPAC study.
- Cohort consisted of non-resectable patients undergoing palliative treatment with gemcitabine or FOLFIRINOX.
- 640 longitudinal plasma samples were analyzed for *KRAS* mutational burden in circulating tumor (ctDNA) using the Trovagene quantitative *KRAS* ctDNA assay.
- Potential clinical utility of ctDNA *KRAS* burden to predict patient survival and treatment outcomes was investigated.
- Time points assayed: baseline, before cycle 2 of chemotherapy, and subsequent samples collected approximately every 2-3 months at time of CT scans..

Methodology

***KRAS* ctDNA assay**

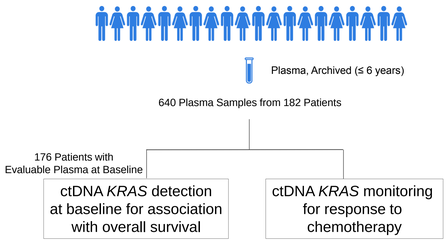
- We developed a highly sensitive mutation enrichment assay for the detection of *KRAS* codon 12/13 mutations in highly fragmented plasma ctDNA.
- The assay is comprised of a mutant allele enrichment PCR step, followed by massively parallel deep sequencing using MiSeq.
- To achieve greater sensitivity in fragmented ctDNA, the enrichment PCR assay utilizes a 31bp footprint. The assay selectively amplifies mutant DNA fragments by using kinetically-favorable binding conditions for a WT blocking oligonucleotide to suppress wild-type (WT) sequence amplification.
- Barcoded adaptor primers are added for compatibility with massively parallel deep sequencing.
- Following sequencing, a proprietary analysis algorithm allows accurate quantitation of input level of mutant DNA. Results are standardized by reporting number of copies detected per 105 genome equivalents (GE).

Statistical analysis

- Associations between clinical variables and OS were investigated using Kaplan-Meier curves, Cox proportional hazards models, and the Extended Cox model with time-dependent covariates (Fisher and Lin 1999; Marubini and Valsecchi 1995).
- A previously determined *KRAS* count threshold of 5.5 copies/100K genome equivalents (GE) was used. All analyses were conducted in R (R Core Team 2014).

Clinical Study Design

Prospective study with retrospectively analyzed samples of 182 patients with non-resectable, locally advanced or metastatic pancreatic cancer undergoing treatment with chemotherapy

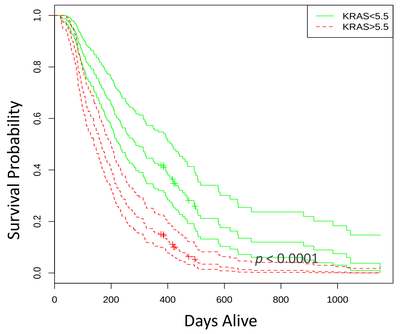


- 640 plasma samples were evaluable (archived for ≤ 6 years).
- 176 out of 182 patients had evaluable baseline samples.
- *KRAS* mutational load in 176 baseline and 640 longitudinal plasma ctDNA samples was analyzed for association with the overall patient survival (OS).

Results

Association between Baseline ctDNA *KRAS* Counts and Overall Survival

- Baseline *KRAS* mutational load in 176 patients was analyzed for association with OS using a Cox proportional hazards model.
- Statistically significant negative association between baseline *KRAS* counts and OS was observed indicating that patients with lower *KRAS* counts survive longer.



Estimated survival curves for patients with baseline *KRAS* counts above and below 5.5 copies/100K GE demonstrate that patients with lower ctDNA *KRAS* burden survive longer (p<0.0001). Estimated survival curves and 95% CI are shown.

Baseline *KRAS* count	N	Chemotherapy	Median survival	95% CI for median survival
≤ 5.5	44	Gemcitabine	296 days	212 – 400 days
> 5.5	103	Gemcitabine	148 days	127 – 180 days
≤ 5.5	15	FOLFIRINOX	499 days	252 – N.E. days
> 5.5	14	FOLFIRINOX	210 days	185 – N.E. days

The median survival rates are statistically significantly different in patients categorized into two groups (*KRAS* ≤ 5.5 copies/100K GE, > 5.5 copies/100K GE) ($p < 0.0001$, the log-rank test).
The hazard rate (HR) of death of the > 5.5 copies/100K GE *KRAS* group is 2.2 (95% CI: 1.5 to 3.1) times as high as the HR of the ≤ 5.5 copies/100K GE *KRAS* group. (N.E., Not estimable). The HR for patients on gemcitabine is 1.7 times as high (95% CI: 1.1 to 2,7) as those on FOLFIRINOX. *N= 176.*

Association between Longitudinal ctDNA *KRAS* Counts on Chemotherapy and Overall Survival

- *KRAS* mutational load in 176 patients with serially collected plasma samples was analyzed for association with OS using the Extended Cox model with time-dependent covariates (Fisher and Lin 1999; Marubini and Valsecchi 1995).
- High *KRAS* counts during treatment were significantly associated with lower OS (p-value <0.001 for continuous and dichotomous *KRAS* variables). The strength of this association was greater for post-baseline *KRAS* counts than for the baseline *KRAS* (p=0.07 vs. p=0.03 in the dichotomous multivariate model). The concordance of time-dependent *KRAS* was 71%, higher than the concordance of baseline *KRAS* in the non-time-dependent model (68.5%).

Variable	Estimate	Standard Error	Hazard Ratio (95% CI)	p-value
Post-baseline *KRAS* > 5.5 cps/100K GE	0.886	0.169	2.4 (1.7, 3.4)	<0.0001
Chemotherapy (Gemcitabine)	0.658	0.235	1.9 (1.2, 3.1)	0.005

- Parameter estimates in the Extended Cox model with time-dependent *KRAS* counts. N=176.

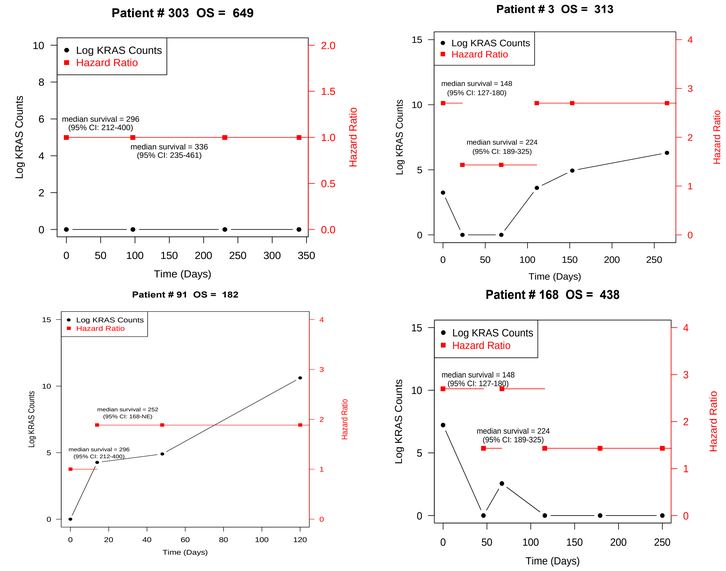
Results

Monitoring *KRAS* ctDNA Burden on Chemotherapy

- Baseline and post-treatment *KRAS* ctDNA mutational load in 136 patients was analyzed for association with OS using a Cox proportional hazards model. Patients were stratified into 4 groups based on *KRAS* signal at baseline and after 2 cycles of chemotherapy (R1).
- The time-dependent *KRAS* ctDNA analysis model tailors predictions of outcomes based on the treatment after baseline.

Baseline *KRAS* cps/100K GE	R1 *KRAS* cps/100K GE	N	Median survival after R1	95% CI for median survival (after R1)	HR (95% CI)
≤ 5.5	≤ 5.5	28	336 days	235 – 461 days	
≤ 5.5	> 5.5	10	252 days	168 – N.E. days	1.3 (0.65 – 2.8), p=0.43
> 5.5	≤ 5.5	20	224 days	189 – 325 days	1.7 (0.94 – 3.1), p=0.08
> 5.5	> 5.5	78	134 days	104 – 159 days	3.4 (2.0 – 5.2), p<0.0001

Longitudinal dynamics of KRAS ctDNA burden and changes in HR and predicted survival after 2 cycles of chemotherapy



Conclusions

- We developed a quantitative PCR-NGS mutation enrichment assay for the detection of *KRAS* codon 12/13 mutations in highly fragmented plasma ctDNA.
- In a prospective study with retrospectively analyzed samples of 182 patients with locally advanced or metastatic pancreatic cancer, a statistically significant negative association was found between baseline ctDNA *KRAS* counts and OS ($p < 0.0001$), indicating that patients with lower *KRAS* burden in ctDNA survive longer.
- Combination of baseline and longitudinal ctDNA *KRAS* burden on chemotherapy was a better predictor of patient outcomes than baseline ctDNA *KRAS* alone ($p < 0.0001$ for post-baseline *KRAS* in the model).
- The time-dependent *KRAS* ctDNA analysis tailors predictions of outcomes based on the treatment and may be clinically useful for treatment management decisions in non-resectable patients with pancreatic cancer.

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