Comparative Circulating Tumor DNA Levels for KRAS Mutations in Non-Reseetable Pancreatic Cancer Patients

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Background
Patients with non-resectable advanced/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.

Analysis of circulating tumor DNA (ctDNA) levels may have several advantages over the use of tumor tissue.

1. No need to obtain invasive surgery.
2. Tumor burden can be monitored over time.

CTDna levels correlate with tumor burden. We aimed to develop a stable PCR assay to detect and monitor KRAS mutations in ctDNA.

Methods
CTDNA was isolated from archived plasma of patients with metastatic pancreatic cancer enrolled in the Danish BIOPAC study. The assay is comprised of a mutant allele enrichment PCR step followed by quantitation of input level of mutant DNA. Results are standardized by parallel deep sequencing.

Analytical Sensitivity: Single copy detection LOD < 0.0005% mutant DNA in a background of wild-type DNA.

Analytical Specificity: 100%.

Lower Limit of Detection (0.0055%)

To demonstrate that the KRAS ctDNA assay has a true single copy detection ability, DNA blends with defined mutant spike in levels of 10, 20, and 40 copies were distributed over 20 wells to obtain 0.5, 1, and 2 mutant copies/well, respectively.

The presented number of mutant copies present in a perfect Poisson distribution with 2 replicates at each mutant copy spike-in level (0.5, 1, and 2) is indicated.

The actual positive/negative hit distribution very closely follows the theoretical model for a Poisson distribution, demonstrating the single copy sensitivity of the assay.

Standard Curves for Quantitation

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 and selectively amplifies mutant DNA fragments while suppressing wild type (WT) sequence amplification using kinetically favorable binding conditions for a WT blocking oligonucleotide.

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 10^6 genome equivalents (GE).

Clinical Study Design

Archived plasma samples (up to 6 years) were prospectively collected from 182 patients with locally advanced or metastatic pancreatic cancer in the Danish BIOPAC study.

The 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 DNA (ctDNA) using the Tro-Mogene quantitative KRAS ctDNA assay.

Potential clinical utility of ctDNA KRAS burden to predict patient survival was investigated.

Timepoints assayed: baseline, before cycle 2 of chemotherapy, and subsequent samples collected approximately every 2-3 months.

Standard curves were developed for each mutation using 288 independent enrichment reactions per curve with different amounts of spiked ctDNA input from 0-500 copies. Master standard curves for KRAS Exon 2 codons G12A/C/D/R/S/V and G13D are shown below.

Conclusions
We developed a quantitative PCR-QSS mutation enrichment assay for the detection of KRAS codon 12/13 mutations in highly fragmented plasma ctDNA. The ctDNA assay has a single copy sensitivity and the ability to detect 0.0055% mutant DNA in a background of WT DNA.

In a prospective retrospective study of 182 patients with 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.

Ability to monitor ctDNA KRAS burden over time is demonstrated.

Longitudinal monitoring of ctDNA KRAS burden over time indicated that patients with longest survival were usually associated with a continued low copy number of ctDNA KRAS mutations over time.

Stratification at baseline of non-resectable pancreatic cancer patients by ctDNA KRAS mutation copy number may be clinically useful for prognosis.