Ultra-Sensitive Detection of Mutant *KRAS* in Circulating Tumor DNA Predicts Survival in Resectable Pancreatic Adenocarcinoma

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Background

- Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer-related death in the United States. Liquid biopsy is an emerging technology with the potential to improve PDAC prognosis and management.
- Mutant *KRAS* is present in 90-100% of PDAC and is a known identifier of aggressive disease, with over 90% of mutations being exon 2 codon 12 variants (G12), followed by codon 13 (G13) and codon 61 (Q61) variants.
- Despite their high prevalence, *KRAS* mutations are often low-frequency in circulating tumor DNA (ctDNA), making accurate detection at standard sequencing depths difficult. The benefit of mutational analysis at ultra-deep sequencing depths remains to be fully understood.

Study Objectives

1. To assess detection and prognostic capability of mutant *KRAS* ctDNA in localized PDAC prior to treatment initiation.

2. To understand the role and potential benefits of conducting mutational analysis at 100,000x sequencing depth as compared to a standard sequencing depth of 20,000x in the analysis of small-volume ctDNA with low-frequency mutations such as *KRAS*.

Results

1. Pertinent mutations in *KRAS* (G12/G13/Q61) were detected in over one-third of our cohort, with the PredicineCARE Ultra panel improving detection by 46% compared to the PredicineCARE panel (16 patients vs 11 patients).
2. Detectable mutant *KRAS* G12 in baseline ctDNA by standard-depth sequencing was significantly predictive of worse survival (median OS of 17.2 months vs NR, $p = 0.0063$; Fig. 2).
3. Detectable *KRAS* mutation by ultra-deep sequencing was highly predictive of worse survival (median OS 16 months vs NR, $p = 0.031$; Fig. 4), whereas detectable *KRAS* mutation by standard-depth sequencing was not ($p = 0.074$; Fig. 1).
4. Co-occurring mutant *KRAS* G12 and Q61 by standard-depth sequencing was significantly predictive of worse survival (median OS 13.2 months vs NR, $p = 0.022$; Fig. 5). Co-occurrence of *KRAS* G12 and Q61 or KRAS G12/Q61/G13 mutations was not strongly predictive of survival ($p = 0.11$; Fig. 3)

Results – Kaplan-Meir Survival Estimates

Fig. 1 Any *KRAS* Mutation by Standard Care Panel

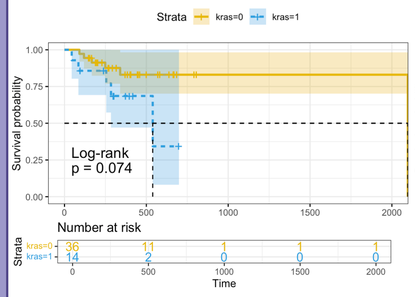


Fig. 2 *KRAS* G12 Mutation by Standard Care Panel

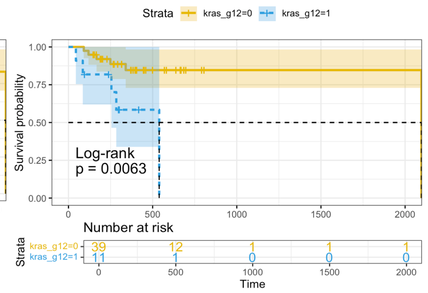


Fig. 3 Co-occurring *KRAS* G12/Q61/G13 or *KRAS* G12/Q61 Mutations by Ultra Panel

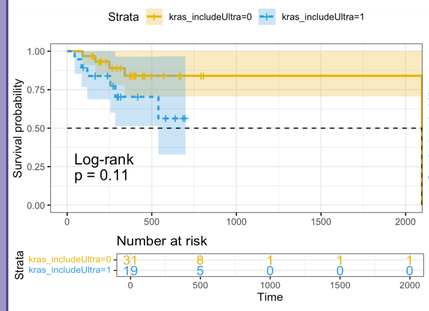


Fig. 4 *KRAS* G12 or G13 Mutation by Ultra Panel

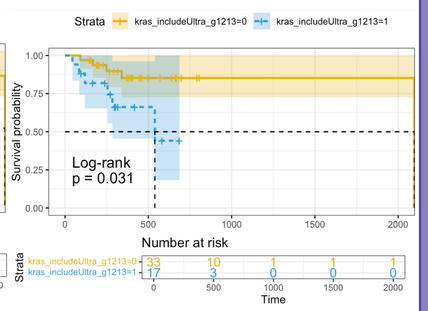
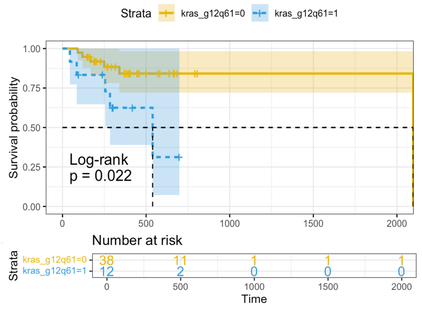
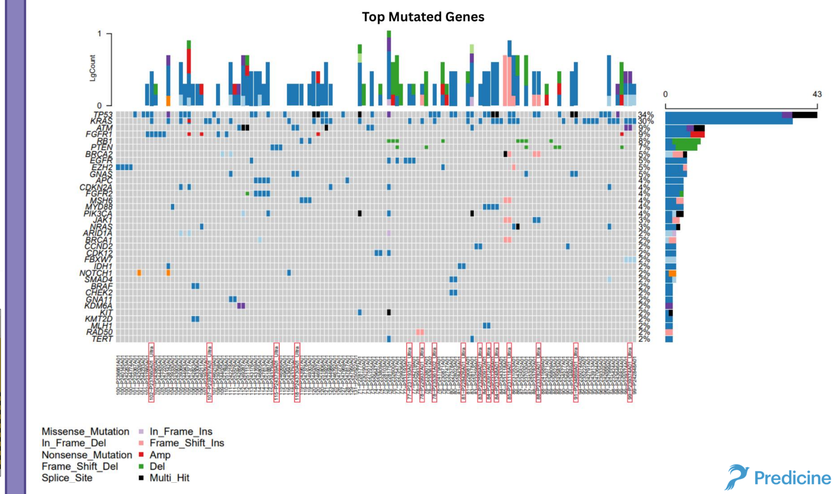


Fig. 5 Co-occurring *KRAS* G12 and Q61 Mutations by Standard Care Panel



Results – *KRAS* Detection and Mutational Landscape

Detectable *KRAS* mutation by Standard CARE Assay	Detectable *KRAS* G12 mutation by Standard CARE Assay	Detectable *KRAS* G12 and Q61 mutation by Standard CARE Assay	Detectable *KRAS* mutation by ULTRA Assay	Detectable *KRAS* G12 and/or G13 mutation by ULTRA Assay
24.4% (11 patients)	17.8% (8 patients)	20% (9 patients)	**35.6% (16 patients)**	33.3% (15 patients)



Conclusions

1. Mutant *KRAS* is detectable in ctDNA of a large portion of patients with localized PDAC prior to treatment initiation.
2. Pertinent *KRAS* mutations (G12/Q61/G13) harbored in ctDNA of patients with localized PDAC are highly prognostic of worse survival.
3. Ultra-sensitive liquid biopsy assays, such as the PredicineCARE Ultra panel, demonstrate robust detection of pertinent *KRAS* mutations in ctDNA beyond that of standard depth assays.
4. Employment of ultra-deep sequencing in liquid biopsy supports improved prognostic stratification, demonstrating potential for improving outcomes for patients with localized PDAC.