Molecular profiling of advanced pancreatic cancer (PC) patients from a phase I/II study using circulating tumor DNA.

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Background
Pancreatic Cancer (PC) has a poor prognosis with a 5-year survival of 9%. Targeted therapies have yet to demonstrate improved outcomes in this disease. Circulating tumour DNA (ctDNA) may be used as a non-invasive method for the detection and quantification of genomic abnormalities. We performed a retrospective-prospective study to assess molecular alterations in the ctDNA of advanced PC patients.

Objectives
- Determine ctDNA molecular mutation profile in patients with advanced PC
- Explore correlation of patient outcome to ctDNA profile
- Explore correlation of patient outcomes to ctDNA mutation allele frequency (AF) and mutant molecules

Materials and Methods
Plasma samples were banked from patients enrolled in the previously reported Phase Ib/II trial of gemcitabine with placebo or vismodegib. Eligible patients had unresectable PC and no prior therapy for metastatic disease. Available patient samples (<3ml) collected pre-treatment and at regular intervals were stored for ~6-8 years were analyzed using InVision assay (enhanced tagged-amplicon sequencing) for “hotspot” regions of 34 genes, including KRAS (exons 2 and 3), and select full gene coverage.

Analyses performed:
- Cox regression analysis on patients stratified by ctDNA mutation profile
- Pearson’s product-moment correlation of allele fraction (AF) to time to progression

Results
- Cohort of 90 patients (Table 1)
- Baseline plasma ctDNA profiling completed in 69 pts
- At least one genomic event was detected in 88.4% of patients (Figure 1)
- Majority of mutations identified were S/NV or indels:
  - KRAS mutations were detected in 54 (78.2%) pts
  - 2 cases presented with IDH1 point mutations
  - 2 individuals with an ERBB2 amplification and FGFR2 amplification
  - Range of 0.07%-24.5% allele fraction (AF) with 20.7% detected at ≤0.5% AF

cDNA Mutation Profile vs patient outcomes
- No significant difference in PFS or OS between patients with pre-treatment KRAS mutations vs those without (p-value=0.669) Figure 3 (OS not shown).
- Patients with concurrent KRAS/TP53/CDKN2A mutations have significantly poorer PFS (p-value = 0.00974) Figure 4.
- Small cohort size requires caution for interpretation

cDNA baseline mutation AF vs patient outcomes
- MAF but did not demonstrate a significant correlation to predict PFS (p-value=0.5696) (Figure 5) or OS (p-value=0.6458) (Figure 6)

Conclusions
ctDNA analysis of this cohort of banked PC plasma samples described the landscape of genomic aberrations at baseline and over time, including rare but potentially important actionable events including ERBB2 and FGFR2 amplifications. We demonstrate a sensitive method for re-analysing trial outcomes, despite limiting plasma volume and time lapse since samples were collected.

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Population (n=90)</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>60 (21-90)</td>
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<tr>
<td>Sex</td>
<td>Male 80 (88.9%)</td>
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<tr>
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<td>Female 10 (11.1%)</td>
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<tr>
<td>Race</td>
<td>White 66 (73.3%)</td>
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<td>Black or African American 12 (13.3%)</td>
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<td>Unknown 2 (2.2%)</td>
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<td>Disease</td>
<td>Pancreatic 35 (38.9%)</td>
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<td>Gastrointestinal 14 (15.6%)</td>
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<td>Other 41 (45.6%)</td>
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<td>Progression-free survival (PFS)</td>
<td>Median (month) 3.4</td>
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<tr>
<td>Overall survival (OS)</td>
<td>Median (months) 6.2</td>
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Figure 1. Distribution of ctDNA mutations by gene

Figure 2. Main ctDNA mutation profiles by patient

Figure 3. ctDNA Mutation Profile vs PFS

Figure 4. ctDNA Mutation Profile PFS

Figure 5. ctDNA baseline mutation AF vs PFS

Figure 6. ctDNA baseline mutation AF vs OS