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2025 ASCO ANNUAL MEETING

Live true single-circulating tumor cell comprehensive genomics unveils clonal evolution and tumor heterogeneity in pancreatic cancer management

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BACKGROUND

Genomic profiling using traditional tissue and ctDNA biopsies have limitations in pancreatic ductal adenocarcinoma (PDAC) due to sampling bias and the inability to capture tumor evolution. While ctDNA enables treatment decisions, longitudinal monitoring for recurrence or therapeutic response, and real-time tumor assessment of tumor evolution, is often constrained by low sensitivity, frequently resulting in no mutation detected (NMD) outsomes. In contrast, single circulating tumor cell (sCTC) genomics offers higher sensitivity but faces challenges in selectively capturing live sCTCs without leukocyte contamination. We address these gaps by identifying clonal and rare sub-clonal mutations in PDAC using 1Cell.Ai's OncoIncytes platform that captures and releases live sCTCs, enabling true single cell comprehensive genomic profiling (CGP), and is further integrated with ctDNA analysis.

PROBLEM STATEMENT

PDAC has poor outcomes due to late detection, aggressive biology, and limited treatment options. Tumor heterogeneity and evolving mutations further complicate therapy, while tissue biopsies often fail to capture this complexity. Non-invasive CTC analysis offers a promising alternative. This study evaluates the value of serial single cell CTC genomic profiling to uncover tumor heterogeneity and monitor clonal evolution & resistance under treatment pressure.

METHODS

Between April 2024 and May 2025, 15 advanced PDAC patients receiving SOC were accrued under MCW IRB approved protocol (PREDICT-MCW NCT ID: NCT05802069). All patients gave consent to investigational interventions. Live sCTCs were isolated at baseline (BL) and follow-up (FL, every three-months, up to 12 months) using 1Cell's OncoIncytes platform from 10 mL of blood. Live CTCs were captured using glass beads with anti-EpCam/anti-transferrin antibodies and individual sCTC was isolated for single cell genomics. DNA from individually captured sCTCs was linearly amplified, followed by target enrichment using OncoIndx 1080 gene CGP assay. Sequencing libraries were prepared and sequenced on Illumina's NovaSeq X Plus (500× depth). ctDNA underwent deeper sequencing at 10,000x coverage. Data was processed using iCare software for sequence alignment & variant calling (Figure 1).

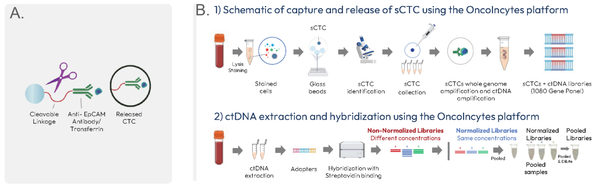

Figure 1. OncoIncytes platform: (A) Live sCTC capture and release shcematics. (B) sCTC isolation workflow from patient blood

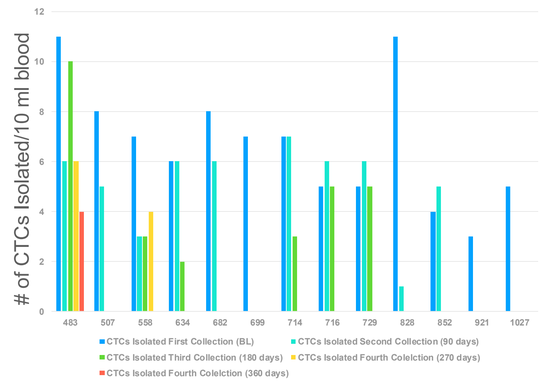

Figure 2. Number of CTCs captured and isolated longitudinally from pancreatic patient samples.

Table 1. Summary of the CTCs isolated from pancreatic cancer patients from the study cohort.

CA Pancreas	
N	34
Patients	15
Mean sCTC distribution	7 cells/10 mL of blood

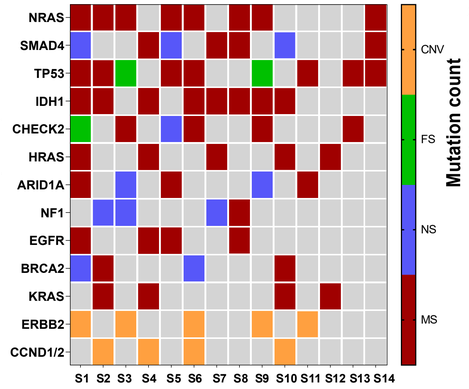

Figure 3. Mutational heatmap showing prevalence in CTC mutations at the sample level.

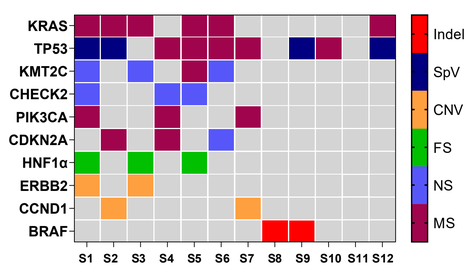

Figure 4. Mutational heatmap showing prevalence of ctDNA mutations at the sample level.

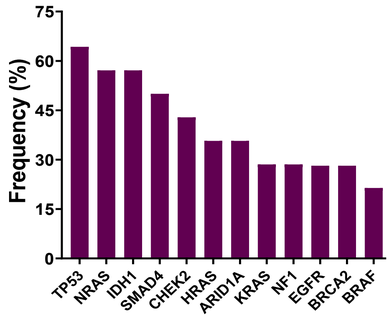

Figure 5. Mutational prevalence in sCTC population.

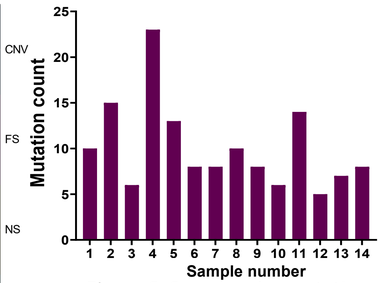

Figure 6. Sample-wise mutation count based on CTC genomics

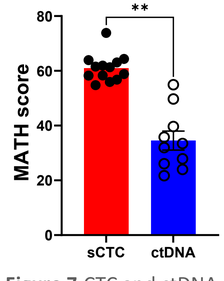

Figure 7.CTC and ctDNA heterogeneity comparison

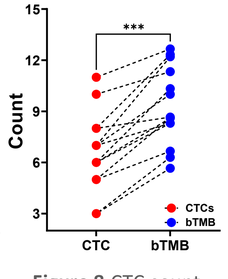

Figure 8.CTC count and bTMB correlation

RESULTS

- Prospectively, 188 live sCTCs were isolated at baseline and follow up (mean sCTC distribution 7) and post SOC treatment, 50 % of the patients exhibited a 30% reduction in sCTC count at FL (Figure 2).
- TP53 mutations were the most frequent alterations observed in sCTCs (64%), followed by NRAS (57%), IDH1 (57%) and SMAD4 (50%) (Figure 3). While paired ctDNA showed TP53 (64%) and canonical KRAS G12 variants (42.8%) at high prevalence (Figure 4). Additional divergent sub-clonal alterations in HRAS, IDH1, and EGFR were detected in CTCs but not in paired ctDNA (Figure 5).
- Co-occurring NRAS and TP53, or SMAD4, mutations along with ERBB2 amplification, were associated with aggressive disease.
- sCTCs reflected high variation in mutation counts among the population (Figure 6) and exhibited high mutational heterogeneity compared to paired ctDNA (Figure 7, P = 0.003).
- Surprisingly, a strong pairing correlation was observed between the CTC burden and blood tumor mutation burden (Figure 8, P = 0.0001, Wilcoxon rank test).

CONCLUSIONS

- Compared to ctDNA, sCTC CGP revealed heterogeneous molecular profile in PDAC, offering precise insights into tumor heterogeneity, clonal evolution, disease progression, and treatment outcome by using 1Cell's OncoIncytes platform.
- Integrating paired DNA profiling of ctDNA and sCTC DNA using OncoIncytes, may provide an enriched sub-clonal mutational landscape besides classical PDAC hotspot mutations in KRAS and TP53.
- Ongoing analyses aim to evaluate temporal dynamics of CGP using ctDNA and sCTC DNA assay for advancing personalized management of PDAC.

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