

Combined circulating tumour cell (CTC) and circulating tumor DNA (ctDNA) analysis of blood from patients with pancreatic cancer

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Background

The challenge to improve outcomes for patients diagnosed with advanced pancreatic cancer remains very real with only small improvements in median survival gained by the use of systemic chemotherapy and little improvement in 5-year survival over the past decades. The advent of next generation sequencing (NGS) of tumour nucleic acids has opened up the possibility of improving outcomes through personalised therapies selected on the basis of tumour genetics. Whilst NGS biomarkers can be measured in tumour biopsies sampled shortly before and after treatment this is often not practical for ethical or logistical reasons, or simple lack of availability. Increasingly liquid biopsies, including **circulating tumour DNA (ctDNA)** and **circulating tumour cells (CTCs)** provide an alternative to determine the genetic profile of cancer patients.

Aims

We set out to: 1) develop methods which will allow combined analysis of both CTCs and ctDNA from a single blood collection tube (BCT); 2) establish a NGS panel for genes frequently mutated in pancreatic cancer; 3) evaluate developed methods by applying them to blood from patients with pancreatic cancer; 4) for ctDNA, compare tumour detection sensitivity using KRAS ddPCR, KRAS NGS and NGS of a panel of 654 genes; 5) compare epitope-dependent (CellSearch) and independent (Parsortix) CTC detection.

Approach

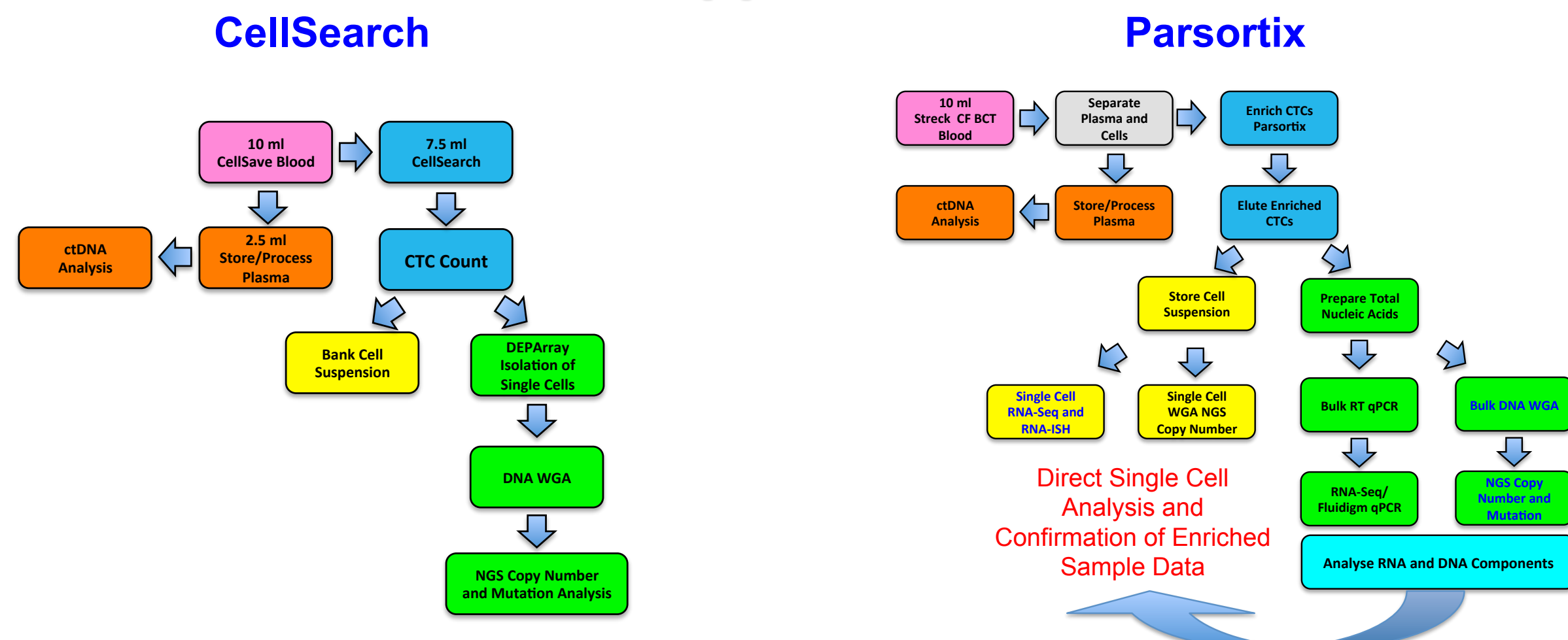


Figure 1. Workflows showing fractionation and analysis of the cellular and plasma components of clinical whole blood samples. Blue text indicates studies in progress.

Results

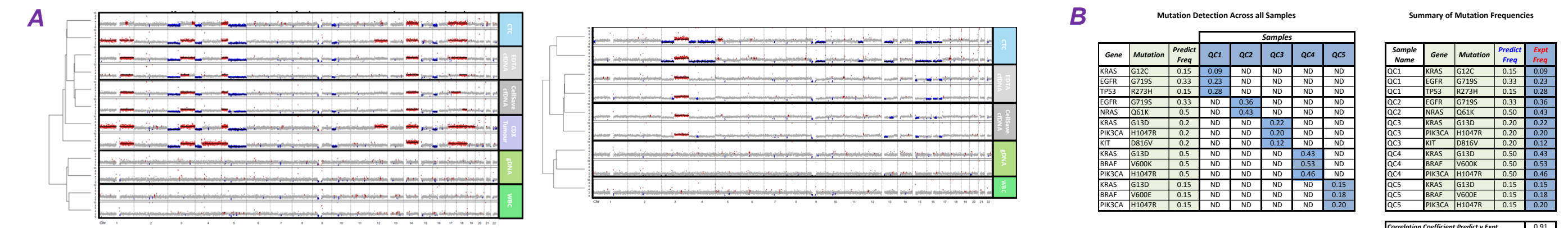


Figure 2. Sensitive ctDNA and CTC NGS analysis methods established. A) shows NGS generated copy number analysis (CNA) of matched single CTCs, EDTA cfDNA, CellSave cfDNA, and CDX tumours from SCLC as recently published¹. Matching patterns of gain (red) and loss (blue) were seen across all related samples. B) External quality assessment samples were used to evaluate an Agilent SureSelect panel of 654 gene chosen primarily on the basis of frequent alterations detected in pancreatic cancer².

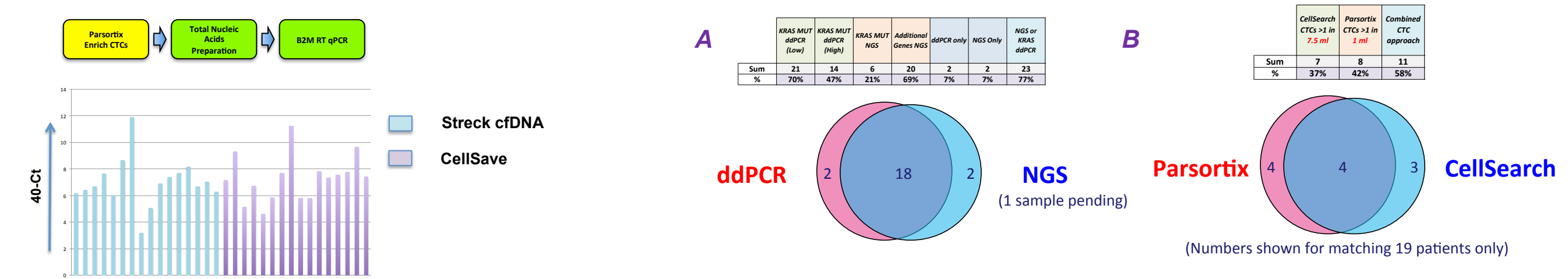


Figure 3. Detection of mRNA in enriched CTCs. RT qPCR of B2M was applied to RNA extracted from Parsortix bulk CTC enrichment of samples from patients with colorectal cancer. Blood collection tubes were maintained at room-temperature for up to 4 days prior to Parsortix enrichment and analysis

Figure 4. Summary of Pancreatic Cancer Patient Pilot Study. A) a comparison of mutant KRAS ddPCR and NGS applied to the same extracted ctDNA samples. B) a comparison of CTCs identified by CellSearch and Parsortix (enriched as previously described³ and CTCs detected in bulk enriched populations by mutant KRAS ddPCR).

Summary and Conclusions

Combined CTC enrichment and cfDNA isolation is readily achievable using a single Streck ctDNA BCT. Results indicate that combined CTC and cfDNA analysis is more sensitive than either approach alone. Initial data indicates presence of RNA in enriched CTCs with more analysis required to establish utility. Developed ctDNA methods also suitable for monitoring response to therapy and identifying mechanisms of resistance⁴.

References

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