Real world results of liquid biopsy in early-stage solid tumors

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BACKGROUND

A "liquid biopsy" is a minimally invasive technique that encompasses plasma based next-generation sequencing (NGS) of circulating cell-free DNA (cfDNA) and cfRNA. Most NGS assays start with silica matrix-based isolation (either spin column or magnetic beads) to extract cfDNA and/or cfRNA. Unfortunately, this extraction process loses 70-80% of genomic material due to binding, washing, and elution steps. As a result, early-stage cancers are often reported as having no detectable cfDNA and cfRNA due to low tumor burden. Here we report initial liquid biopsy results from early-stage solid tumors using the Circulogene Theranostics Personalized Gene Profile (CGP, 50-gene panel) on cfDNA and cfRNA. CGP uses proprietary in situ enrichment which is extraction-free and loss-free and may have advantages over other assays by focusing on upstream starting material processing. CGP uses an input volume of 20 uL plasma for cfDNA and 400 uL for cfRNA per case.

METHODS

DNA mutations detected and, where available, PD-L1 expression, ALK, and ROS1 fusions in RNA were compiled retrospectively from a single center. Turnaround time (TAT) was calculated as the difference in calendar days between the date the sample was received at the vendor to the date of the first NGS report for that sample.

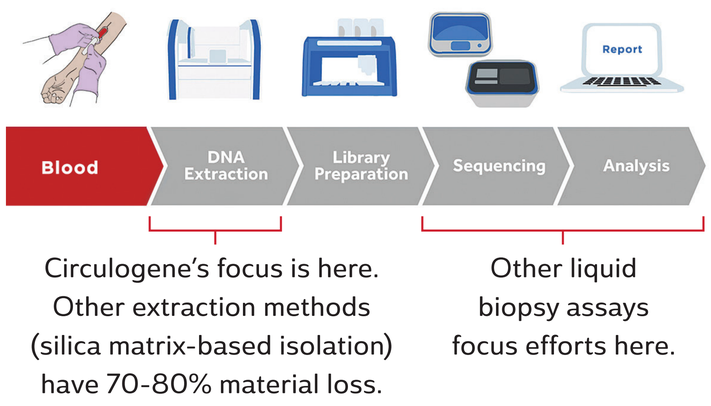
RESULTS

Sixteen patients (median age 67.5 years, range 37-91; 10 men and 6 women), all with stage II or III cancer, underwent testing between December 2015 and November 2017. The majority of cancer types were rectal, non-small cell lung, breast, pancreatic, and prostate. Most common DNA mutations recorded were TP53, KDR, SMAD4, and VHL. There was a median of 4 mutations/patient (range 0-8), with a 0% sample failure rate. Overall 14 cases had detectable mutations (87.5%). Two samples were run when PD-L1 was added to the assay and both were negative. All samples were run within 5 days of collection. Median TAT was 7 days (range 4-15) (Tables I and II).

Table I. Summary of Results	
Median Age (range)	67.5 (37-91)
Gender	10M: 6F
Frequency of Common Gene Mutations*	
TP53	24
SMAD4	4
KDR	3
VHL	3
# Cases Without Mutation (%)	2 (12.5)
Median # of Mutations/Patient (range)	4 (0-8)
*some cases may have multiple mutations in same gene	
Median TAT days (range)	7 (4-15)

Table II. Patient Level Detail

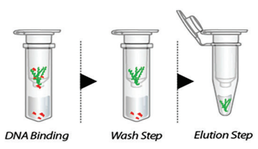
ID#	Cancer Type	Age	Gender	Stage	Results	TAT days
1	Bladder	41	M	2	TP53x4, PI3KCA, KDR, VHL	4
2	Breast	67	F	2	No mutations detected	9
3	Esophageal	91	F	2	No mutations detected	6
4	Non-small cell lung	82	F	2	TP53x4, PTPN11, PD-L1 negative	11
5	Pancreatic	37	M	2	TP53, PTPN11	6
6	Rectal	84	M	2	TP53	7
7	Parotid	68	M	3	BRAF, CTNNB1, FGFR3, SMAD4x2, TP53x3	5
8	Rectal	74	F	3	TP53	4
9	Pancreatic	72	F	2B	EGFR, RET, KDR, SMAD4, VHL	14
10	Non-small cell lung	67	M	3A	SMAD4, ALK, TP53	10
11	Breast	65	F	3B	PI3KCA, TP53, KIT, EZH2	5
12	Non-small cell lung	63	M	3B	KDR, EGFR, TP53x2	15
13	Rectal	60	M	3B	TP53x3, JAK3	7
14	Prostate	63	M	2	BRAF, ERBB4, HNF1A, RB1, PD-L1 negative	9
15	Prostate	73	M	2	TP53x2, MET, PTEN	7
16	Anal	75	M	2 or 3	TP53, VHL, ATM, APC	9



Circulogene's focus is here. Other extraction methods (silica matrix-based isolation) have 70-80% material loss.

Other liquid biopsy assays focus efforts here.

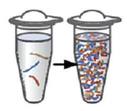
In Vitro Silica Extraction/Concentration



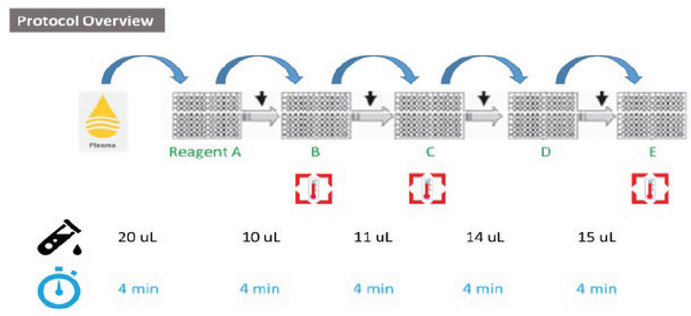
X Unavoidable sample loss
X Higher prevalence of failed sequencing
X Labor-intensive processing
X Large volume reduces ability to automate efficiently
X Wastes valuable biobank samples (2–5mL) for a single sequencing

vs.

***In Situ* Circulogene Enrichment**



✓ **97% success rate**
✓ **Near-full cfDNA recovery**
✓ **Less labor intensive**
✓ **Easily automated and high-throughput in a benchtop footprint**
✓ **Prolong and extend biobank sample usage (20–160uL)**



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CONCLUSIONS

This real world analysis in early-stage cancer indicates that despite the low plasma sample input, there were no sample failures, and most cases had detectable mutations, suggesting CGP in situ enrichment is robust. More experience with this assay in early-stage disease with linkage to disease recurrence outcomes after definitive therapy is warranted.

ACKNOWLEDGMENTS

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