Cancer patient paired congruence between a commercially available tumor tissue and a liquid biopsy NGS assays

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BACKGROUND

Next-generation sequencing (NGS) assays are increasing being used in oncology clinical practice. With the recent FDA approval of a commercially available tumor tissue NGS assay and reports of low concordance across different platforms, we sought to evaluate the concordance of DNA mutations and PD-L1 testing results from Caris Molecular Intelligence® testing (CMI, initially 40 then 592-gene panel) on tumor tissue and Circulogene Theranostics Personalized Gene Profile (CGP, 50-gene panel) on cell-free circulating DNA and RNA.[1-4]

METHODS

We conducted a single center retrospective analysis of stage 4 solid tumor patients that had matched paired testing. DNA mutations detected by either assay and, where available, PD-L1 IHC (CMI) and PD-L1 mRNA (CGP) were compiled to assess for concordance. A Student's t-test or Chi Square test was applied to DNA mutation calculations. 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 and excluded quantity not sufficient (QNS) reports.

RESULTS

Thirty-seven patients (median age 69 years, range 38-86); 14 men and 23 women) underwent both CMI (between June 2014 and September 2017) and CGP (between November 2015 and August 2017) testing. The majority of cancer types were breast, non-small cell lung, colon, and pancreatic cancer. The median time from tumor sample collection to report date was 59 days (range 11-959 days). Most common DNA mutations recorded by either assay were TP53, PI3KCA, KRAS, APC, and PTEN. CMI had a median of 1 mutation/patient (range 0-4), with 3 QNS reports (8% failure rate). CGP had a median of 3 mutations/patient (range 0-11), with no QNS results (0% failure rate). The total number of mutations reported for CMI and CGP were 48 and 148 ($p<0.0001$), respectively (Table I). Overall concordance at the gene level was 14/196 (7.1%). None of the mutated genes were at the same exon. There were four paired samples with PD-L1 results, and concordance was 3/4 (75%) (Table II). All CGP samples were run within 5 days of collection. When limiting analysis to paired cases with CMI run on tumor sample that was collected within the preceding 30 days (n=10), CMI had a median of 1 mutation/patient (range 0-4), while CGP had a median of 1.5 mutations/patient (range 0-9). The total number of mutations reported for CMI and CGP were 16 and 34 ($p>0.05$), respectively. Overall concordance at the gene level was 2/50 (4.0%). TAT was available for 29 pairings of CMI and CGP testing results, and CGP reported first results for 19 of these (65.5%). CMI had a median TAT of 10 days, while CGP has a median TAT of 8 days. Four cases had samples collected and reported by CMI and CGP within 30 days of each other showing concordance of 14.8% (Table III, Figure).

Table I. Comparison of Platform Results			
Median Age (range)	69 (38-86)		
Gender	14M: 23F		
Cancer Type (%)			
Breast	9 (24.3)		
Non-small cell lung	6 (16.2)		
Colon	5 (13.5)		
Pancreatic	5 (13.5)		
	CMI	**CGP**	
Frequency of Common Gene Mutations*			
TP53	11	46	
PI3KCA	6	6	
KRAS	5	2	p=0.0002
APC	6	2	
PTEN	2	14	
Median # of Mutations/Patient (range)	1 (0-4)	3 (0-11)	
Total # of Mutations (n=37 patients)	48	148	p<0.0001

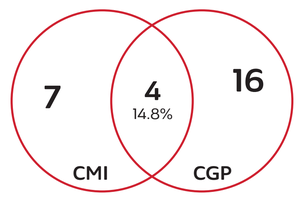
*some cases may have multiple mutations in same gene

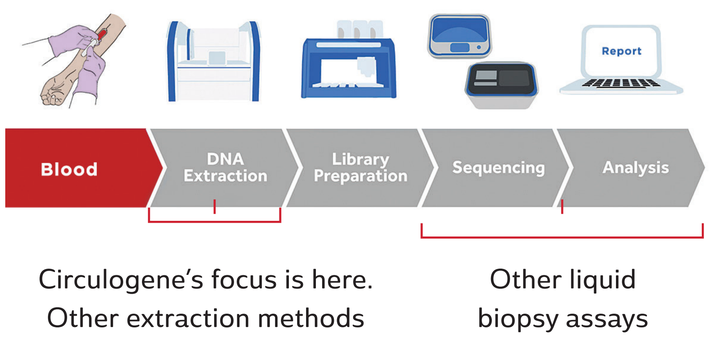
Table II. PD-L1 Results	CMI	CGP
PD-L1 results	n=32	n=5
+ IHC (%)	4 (12.5)	-
+ mRNA (%)	-	1 (20.0)
Concordance n=4 available	0 IHC positive	1 mRNA positive
Median TAT days (range)	10 (7-8)	8 (4-27)

2 Commercially available NGS assays
Tumor and liquid biopsy
Concordance at gene mutation and PD-L1 level

Mutations found in 4 matched pairs*

*tumor and blood samples collected within 30 days of NGS

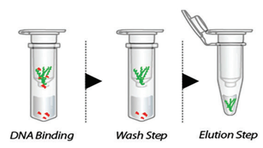




Circulogene's focus is here. Other extraction methods (silica matrix-based isolation) have 70-80% material loss.

Other liquid biopsy assays focus efforts here.

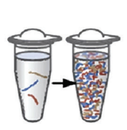
In Vitro Silica Extraction/Concentration



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)**

Protocol Overview

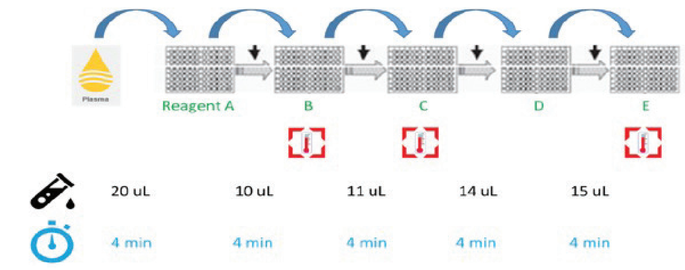


Table III. CMI and CGP collected and reported within 30 days of each other					
Tumor type	**Age**	**Gender**	**# of days apart**	**CMI result**	**CGP result**
ER/PR+ Breast	43	F	19	No mutations, PD-L1 negative	KIT, ATM, IDH2, CTNNB1
Colon adenocarcinoma	62	F	24	KRAS, TP53, PD-L1 negative	BRAF, CTNNB1, PD-L1 negative
Lung adenocarcinoma	69	F	2	EGFRx2, ERBB2, PD-L1 negative	AKT1, ALK, ERBB2, GNASx2, TP53x3, VHL, PD-L1 negative
Pancreatic adenocarcinoma	38	M	7	KRAS, TP53, PD-L1 negative	BRAF, CTNNB1, PD-L1 negative

CONCLUSIONS

This analysis indicates there is a marked difference in concordance between tumor tissue and liquid biopsy NGS assays. This is in agreement with other recent publications evaluating other platforms. While only a limited number of pairs had PD-L1 results, the concordance was high. More DNA mutations were identified by CGP and TAT was faster for the majority of pairings. The results point to the dynamic nature of genomic changes and may suggest that limiting to one test in time may not be ideal.

REFERENCES

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4. Yeh,C-H, Spurgin J, Ford, A, Athanasuleas A, Manne U. Clinical validation of a next-generation sequencing assay specifically for blood-drop liquid biopsy. AACR 2016 Annual Meeting

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