Somatic Mutation Detection from Liquid Biopsies via NGS: Urological Cancers

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Abstract

Introduction: Next generation sequencing (NGS) of blood-derived nucleic acids is an emerging paradigm for determining the mutational status of cancer patients over time. Both circulating tumor cells (CTC) and cell-free circulating DNA have been proposed as possible sample types for extracting tumor DNA. Here we present data from a CTC enrichment modality that results in tumor cell purities of >10% and a high sensitivity NGS data analysis workflow that enables the use of standard amplicon panels typically used for primary tissue. This study is aimed at urological cancers (kidney, prostate), and adds to previously published data presented for bladder cancer patients using this technique.

Results Multisite analytical validation data, based on spiking of cells into whole blood, and a matched molecular and bioinformatics approach demonstrate a detection limit down to 10 cells from a blood draw with a false positive rate of below 0.1 calls per sample. Clinical data from two different urological cancer pilot studies (prostate and kidney) demonstrates the detection of somatic variants for a majority of samples, and significant overlap between detected mutations and known somatic mutation sites. For example, in prostate patients, we detect common mutations (i.e. TP53, PTEN and APC genes) that are similar to the population distribution of mutation rates in tissue biopsies.

Conclusions: This assay makes possible the detection of somatic variants from urological cancer patients without the need for a tissue biopsy.

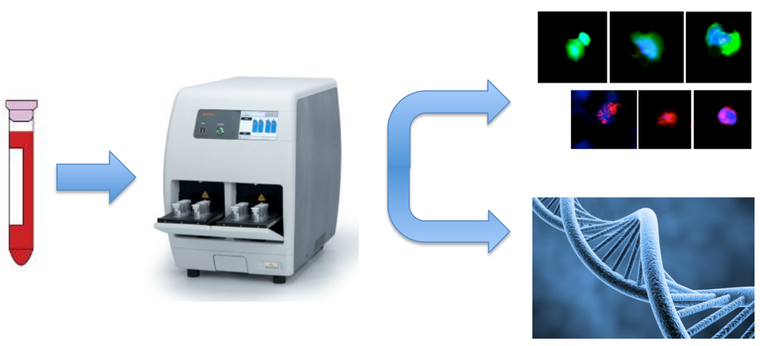

Figure 1: Study Design Outline. All patients had blood drawn for CTC analysis. Half of the sample was processed for enumeration, while a second half had DNA extracted and was put through the IsoFlux DNA workflow. All samples were enriched for CTCs using a proprietary antibody cocktail on the IsoFlux System.

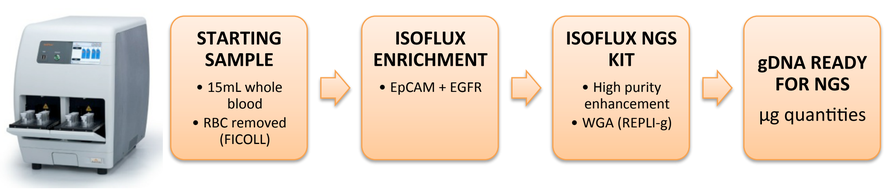

Figure 2. Next generation sequencing workflow. Based on the IsoFlux NGS Kit that allows routine purification of CTC samples to >10% tumor DNA content, a workflow was developed for NGS characterization of patient samples utilizing an commercially available cancer hotspot panels and the PGM sequencing instrument (Life Technologies). CTC enrichment is followed by lysis, DNA amplification, library preparation / sequencing and a specialize variant filtering algorithm.

Chr:Pos	Gene Name	4 Tumor Cell / ml spike (allele freq)	Matched Normal (allele freq)	Gene Region	In COSMIC ?	In dbSNP?
7:140481417	BRAF	11.0%		exon	True	False
12:25398281	KRAS	4.3%		exon	True	False
17:7577099	TP53	14.0%		exon	True	False
3:178917005	PIK3CA	3.7%		intron	False	True
4:55152040	PDGFRA	91.3%	98.2%	exon	True	True
4:1807894	FGFR3	99.8%	99.8%	exon	False	True
10:43613843	RET	96.2%	100.0%	exon	False	True
5:149433597	CSF1R	29.2%	20.9%	UTR3	False	True
19:1220568	STK11	53.8%	56.8%	intron	False	False
10:43615633	RET	43.9%	50.0%	exon	True	True
11:534242	HRAS	37.6%	45.2%	exon	True	True
13:28602292	FLT3	42.0%	45.4%	intron	False	True
7:128846063	SMO	1.9%	1.9%	exon	False	False

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4:55152040	PDGFRA	91.3%	98.2%	exon	True

Legend:
homozygous
heterozygous
likely somatic
likely false positive

Figure 3: Example analysis results - analytical samples Using a mesenchymal-like cell line (MDA-MB-231) the limit of detection of the CTC-based NGS was determined by spiking cells into 14mL of healthy donor blood, recovery and blinded NGS analysis to discover the variants present. The detection limit is below 2 cells / ml blood for all variants, with a very low false positive rate (1 call in 18 samples tested) and good site-to-site reproducibility). A sample analysis is shown above: the left panel shows all calls for both the tumor cell and matched normal samples. Matched normals consist of white blood cells remaining after the CTC separation, and obtained from the same blood draw. All of the germ line mutations are present in both the CTC and matched normal samples (8 calls); somatic mutations present in the CTC sample only are assembled into a final reported set (right panel).

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Disclosures
C. Ionescu-Zanetti: Employee of Fluxion Biosciences Inc, CA; M. Schwartz: Employee of Fluxion Biosciences Inc, CA. All other authors have declared no conflicts of interest.

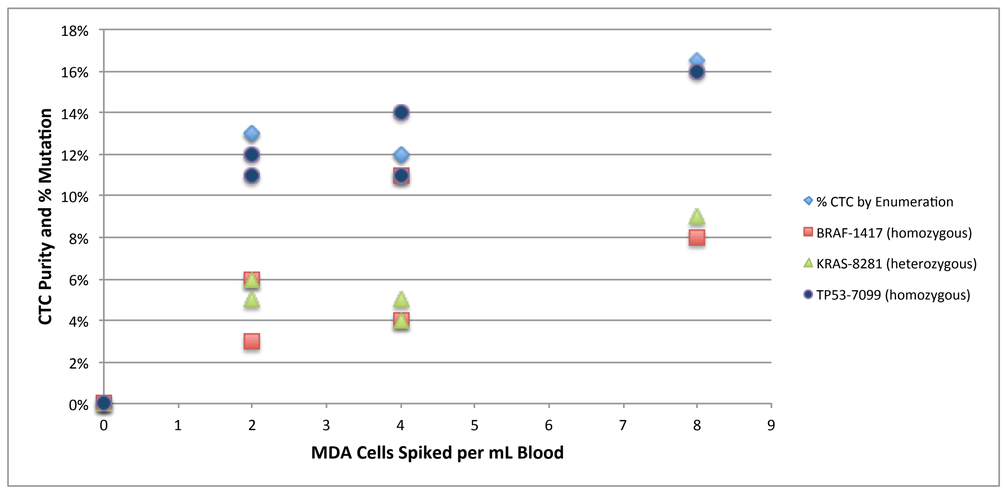

Figure 4: Analytical validation of the CTC to NGS assay Using a mesenchymal-like cell line (MDA-MB-231) the limit of detection of the CTC-based NGS was determined by spiking cells into 14 mL healthy donor blood, IsoFlux enrichment, targeted amplification using the CHPv2 panel, and blinded NGS analysis to discover the variants present. The detection limit is below 2 cells / ml blood for all variants, with a very low false positive rate (no false positives in this data set). Note that the CTC purity for matched samples is in the range of 12-16%, with a good match between the expected and detected allele frequency of mutations.

Patient number	Indication	CTC Enumeration	Gene Panel
1	Prostate	121	Oncomine
2	Prostate	38	Oncomine
3	Prostate	43	Oncomine
4	Prostate	1606	CHP v2
5	Prostate	15	CHP v2
6	Prostate	585	CHP v2
7	Prostate	75	CHP v2
8	Prostate	243	CHP v2
9	Prostate	245	CHP v2
10	Renal Cell Carcinoma	248	RCC Panel
11	Renal Cell Carcinoma	14	RCC Panel
12	Renal Cell Carcinoma	731	RCC Panel
13	Renal Cell Carcinoma	525	RCC Panel
14	Renal Cell Carcinoma	230	RCC Panel
15	Renal Cell Carcinoma	320	RCC Panel

HEALTHY CONTROLS	
Patient	CTC COUNT
H1	1
H2	0
H3	2
H4	0
H5	3
H6	4
H7	4
H8	8
H9	2
H10	1
H11	2
H12	0

Figure 5: Patient Samples and Enumeration Results. CTC counts are presented for the 15 patients enrolled in the pilot trial, as compared to a healthy normal cohort. The study consisted of prostate and kidney cancer patients, with enriched CTC samples sequenced at the CompanionDx lab using three different commercially available cancer gene panels. These advanced patients had high CTC counts as compared to healthy baselines.

Chr:Pos	Ref/ Alt	Gene Name	CTC Sample (allele freq)	Matched Normal (allele freq)	Gene Region	Effect	In COSMIC ?
22:30057204	G/A	NF2	0.60%		exon	Missense	False
10:123279540	C/T	FGFR2	0.65%		exon	Missense	False
4:153253775	G/A	FBXW7	1.13%		exon	Missense	False
9:98240453	C/T	PTCH1	1.26%		exon	Missense	False
17:7578230	C/A	TP53	1.61%		exon	Missense	True
5:112175771	G/A	APC	3.30%		exon	Missense	True
11:119149000	C/T	CBL	3.45%		exon	Missense	True
17:7577121	G/A	TP53	13.88%		exon	Missense	True
4:55972974	T/A	KDR	65.92%	48.48%	exon	Missense	True
5:112177171	G/A	APC	93.71%	99.13%	exon	Other	True

Chr:Pos	Ref/ Alt	Gene Name	CTC Sample (allele freq)	Matched Normal (allele freq)	Gene Region	Effect	In COSMIC ?
1:11190646	G/A	MTOR	74.45%	55.11%	exon	Other	True
1:11205058	C/T	MTOR	28.35%	50.01%	exon	Other	True
5:67589188	C/T	PIK3R1	72.26%	47.81%	exon	Other	True

Figure 6: Sample NGS Results - Patient Samples. The vast majority of the CTC-enriched samples processed through the analysis pipeline yielded genomic abnormalities; the two examples shown utilized the Oncomine (left table, patient 1) and RCC (right table, patient 14) panels. While all germ line mutations were present in both enriched CTC and matched normal samples, the abnormalities presented here were present in the CTC sample but not in the normal sample.

Gene Panel	Description	Median No. Variants	COSMIC Variants
Oncomine	Pan-cancer, 130 genes	12	5
CHP v2	Pan-cancer hotspot, 52 genes	1	0.5
RCC	Renal Cell Carcinoma specific, 28 genes	3	1

Figure 7: Median variant numbers by panel. Median variant counts present in CTC samples as compared to matched normal samples were calculated for each of the three oncogene panels used. The larger pan-cancer panel Oncomine (Life Technologies) did an excellent job of detecting abnormalities, while the more limited hotspot panels detected mutations consistent with the panel coverage for these indications (prostate, renal cell carcinoma).

Summary

We present analytical and patient sample validation for a novel approach to molecular profiling of liquid biopsy samples from prostate and renal cell carcinoma patients via NGS.

The methods presented enabled the recovery of high CTC counts in all late stage patients participating in this study, as well as the isolation of matched normal samples by collecting the white blood cell fractions resulting from the same separation. Both CTCs and leukocyte fractions were analyzed using a sensitive sequencing and NGS data analysis pipeline capable of detecting variants down to 0.5% allele frequency with high fidelity. Abnormalities found in the tumor cell samples were not present in matched normals, and were similar to abnormalities typically detected in tissue based studies for similar patient cohorts and gene panels. In relation to other liquid biopsy methods, the high sample purity provided by this workflow enables use of standard genomic panels as opposed to highly specialized sequencing and analysis methods.

This study demonstrates the detection of somatic mutations in urological cancers from a blood draw. Ongoing studies include sequencing of CTCs cultured post enrichment, mutational profile changes over time, and correlation to matched biopsy samples.