Somatic mutation detection via sequencing using circulating tumor cell samples from patients with renal cell and prostate cancer

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Introduction

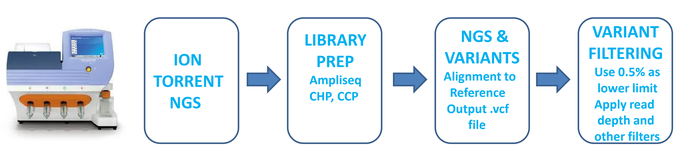
Tumor genotyping using fluid samples such as blood can potentially allow tracking of dynamic changes in mutational profiles over time and allow better access than biopsies. We present a method to detect somatic mutations from a blood draw, where circulating tumor cell (CTC) enrichment above 10% of total cell numbers allows the use of standard gene panels typically used to analyze tissue-based biopsies.

CTC isolation and sequencing uses the IsoFlux™ System (Fluxion)

Summary of study design. Blood draw was analyzed for both CTC enumeration (using proprietary Ab cocktail) and DNA extraction. Extracted DNA was put through the IsoFlux DNA workflow in collaboration with Companion Dx.

Methods

Clinical samples were obtained from 9 patients with metastatic, castration-resistant prostate cancer (mCRPC) patients and 6 with metastatic renal cell cancer (RCC), followed by CTC enrichment using the IsoFlux™ System. Cells were lysed and DNA amplified by whole genome amplification (WGA) using the NGS Kit (Fluxion Biosciences) and quantified via qPCR. CTCs are defined as CK+, CD45- nucleated cells (DAPI+) for cell enumeration. Analytical samples were prepared by spiking tumor derived cell lines into whole blood and parallel analysis (Figure 1). Next-generation sequencing was performed using 3 targeted cancer panels on the Ion torrent™ PGM platform: the Ion ampliseq™ cancer hot spot panel (52 genes; 6 (PC) samples), Oncomine (143 genes; 3 PC samples), and a 29-gene panel of actionable mutations in RCC (6 samples). Data was analyzed using a customized variant calling/filtering pipeline. Variant filtering and functional interpretation was performed using VarSeq™. All data was analyzed in a blinded manner.



Next generation sequencing procedure. A workflow was developed for NGS to characterize patient samples using commercially available cancer hotspot panels and the PGM sequencing instrument (Life Technologies). CTC enrichment is followed by DNA extraction and amplification, library preparation/sequencing and a specialize variant filtering algorithm.

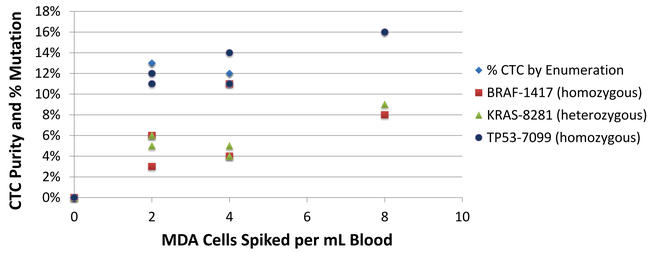


Figure 1. Analytical Validation of the NGS assay using spiked blood. Using a mesenchymal-like cell line (MDAMB-231) the limit of detection of the CTC-based NGS was determined by spiking cells into 14 mL healthy donor blood, IsoFlux enrichment, targeted amplification using the CHPv2 panel, and blinded 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.

Results

Our method was able to isolate CTCs from all patient samples (Table 1). WGA DNA concentrations were at a range of 25-164 ng/µL (median, 69) in PC and 29-180 ng/µL (median, 69) in RCC. CTC purity after the first enrichment step was in 2.9-33.7% (median, 10.5%) of PC samples and 1.9-33% (median, 14.5%) of RCC; final CTC purity is estimated at 5-40%. We found 1 variant/sample using hotspot, 12/samples using Oncomine, and 3/sample using the RCC panel (Figure 2).

Table 1. Summary of CTC enrichment, DNA yield, and sequencing strategy of enumerated renal cell and prostate cancer patients.

Patient #	CTC/Total cell	% Purity	DNA (ng/µl)	Gene Panel
RCC:				
001	248/1522	16.3	78	RCC
002	14/1273	1.1	87	RCC
003	731/35613	2.1	60	RCC
004	529/4170	12.6	32	RCC
005	230/698	33	180	RCC
006	320/1303	24.6	29	RCC
Prostate:				
001	121/359	33.7	62	Oncomine
002	1606/4890	32.8	72	Hotspot
003	15/921	1.6	69	Hotspot
004	585/5262	11.1	54	Hotspot
005	75/715	10.5	47	Hotspot
006	38/180	21.1	25	Oncomine
007	43/524	8	154	Oncomine
008	243/8351	2.9	164	Hotspot
009	245/2780	8.8	140	Hotspot

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 2. Typical 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 (Top, patient 1) and RCC (Bottom, 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.

Conclusion

Our assay consistently detected somatic variants from blood draw using standard gene panels in both PC and RCC (Table 2). Obtaining repeat tumor biopsies from patients during treatment and/or at time of progression is both challenging and impractical from clinical perspective. Our assay provides molecular characterization using standard blood draws. We will expand this research longitudinally using serial samples obtained in parallel with our clinical trials in PC and RCC to determine the molecular alterations that occur during treatment.

Table 2. Summary of variants detection using various gene panels

Gene Panel	Description	Median No. Variants	COSMIC Variants
Oncomine	Pan-cancer, 143 genes	12	5
CHP v2	Pan-cancer hotspot, 52 genes	1	0.5
RCC	Renal Cell Carcinoma specific, 29 genes	3	1

Future Directions

1. Develop program longitudinally to include all PC and RCC patients in clinical trials
2. Improve CTC heterogeneity by including more diverse surface markers in the isolation step
3. Increase tumor % by 3D cell culture