Mutational analysis from circulating tumor cells using next-generation sequencing

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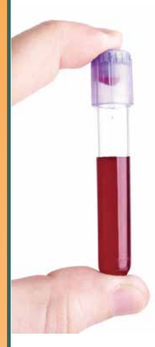
INTRODUCTION

Tumor mutational analysis provides insight into patient drug response, prognosis, and tumor biology. A key limitation to this process is the availability of tumor tissue that adequately represents the current disease status. This study presents a Next Generation Sequencing (NGS) workflow utilizing enriched circulating tumor cells (CTCs) as an input.

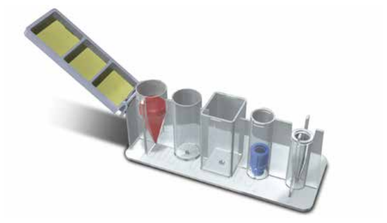
The CTC enrichment is performed using the IsoFlux System that accommodates multiple capture antibody cocktails and has been shown to be effective across multiple indications.

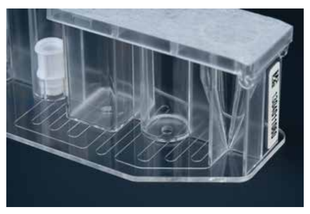
METHODS



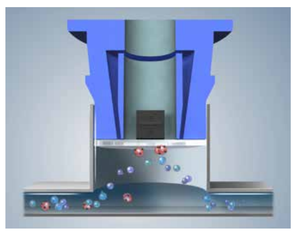
Sample Collection
2 x 10cc EDTA blood tubes were collected and shipped overnight at room temperature.

Bead Coupling
RBCs removed with FICOLL
EpCAM and EGFR magnetic beads added
Sample loaded into microfluidic cartridge

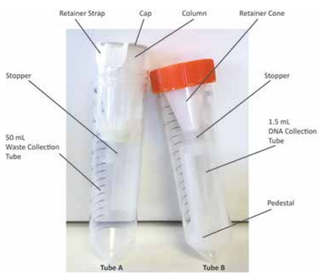




CTC Enrichment
Cells pass through microfluidic isolation zone
Target cells with beads captured on roof of channel
Up to 4 samples/run, 12 samples/day, 4ºC controlled



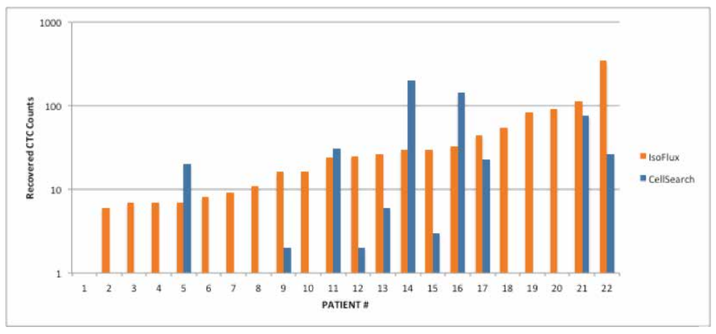
NGS Analysis
CTCs transfered in low volume (5µL)
Purity enhancement with IsoFlux NGS Kit (WGA optional)
Ampliseq Cancer Hotspot v2 library prep (50 genes)
Sequencing on IonTorrent PGM (Thermo Fisher)
Variant calling and filtering using Ion Reporter



ENRICHMENT OF CTCS FROM CLINICAL SAMPLES

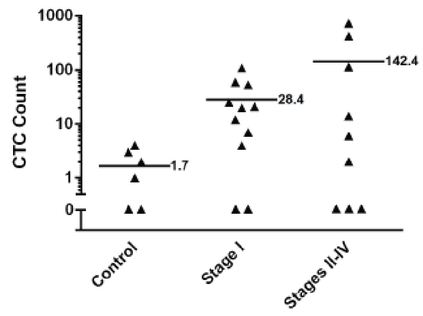
The IsoFlux System has been used to enrich CTC samples from hundreds of cancer patients across multiple indications. With magnetic bead isolation, the IsoFlux System can utilize a variety of surface markers for CTC capture, including EpCAM, EGFR, Her2, and other disease/drug-specific targets. Users can define their own capture cocktails with straight-forward protocols.

Prostate



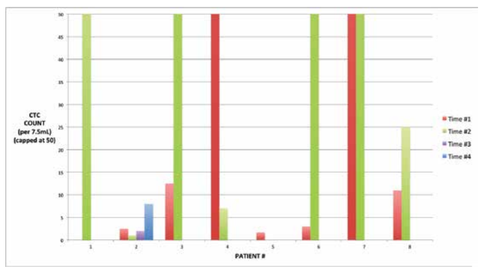
Matched samples from 22 prostate cancer patients were used to compare CTC recovery using the IsoFlux System versus CellSearch platform. IsoFlux yielded 21/22 patients (95%) with CTC counts >4 compared with 8/22 patients (36%) with CellSearch (data previously published J. Translational Oncology).

Lung



20 NSCLC samples were collected (N=11 Stage 1, N=9 Stage 2-4) and 6 healthy donor samples. Cell count differences were statistically significant between control versus stage 1 (p=0.01) (data previously presented by Max Diehn, Stanford University).

Kidney



Blood samples were collected from 8 kidney cancer patients at UT Southwestern pre-surgery (Time #1) and during one or more post-surgery follow-ups.

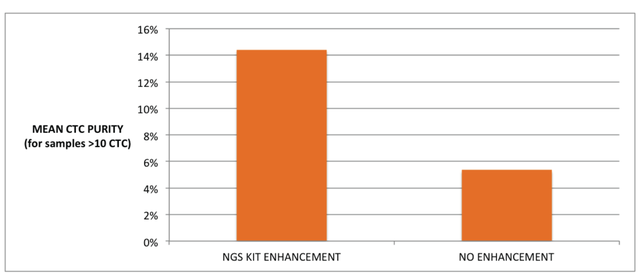
PATIENT	Time #1	Time #2	Time #3	Time #4	pT	pN	DISEASE STATUS
1	N/A	85	0		2	0	NED
2	3	1	2	8	3B	0	AWD
3	13	302			3B	1	AWD
4	435	7	0		3A	0	NED
5	2				BENIGN	BENIGN	NED
6	3	346			3B	0	DOD
7	203	225			3B	0	NED
8	11	25			3B	0	NED
% > 10 CTC	57%	71%	0%	0%			

Low CTC counts (<25) and significant drops in CTCs are highlighted in green.
High CTC counts (>25) and significant increases in CTCs are highlighted in red.
Disease Status: NED = no evidence of disease, AWD = alive with disease, DOD = dead of disease
(all as of last clinical follow up - study still in progress)

NGS ANALYSIS

MDA CELL NUMBER (per mL blood)	CTC PURITY	SITE 1 BRAF	SITE 1 KRAS	SITE 1 TP53	SITE 1 FALSE POSITIVES	SITE 2 BRAF	SITE 2 KRAS	SITE 2 TP53	SITE 2 FALSE POSITIVES
Pure MDA-MB-231 cell line	100%	58%	68%	99%	NA	58%	68%	99%	NA
12	22%	Not tested				29%	22%	44%	
						21%	12%	24%	
8	15%	5.0%	7.0%	11.0%	0	6.0%	6.0%	10.0%	0
		3.0%	3.0%	5.0%	0	3.0%	2.0%	4.0%	0
4	8%	6.0%	6.0%	11.0%	0	7.0%	6.0%	12.0%	0
		ND	6.0%	1.0%	0	ND	5.0%	1.0%	0
2	5%	2.0%	2.0%	6.0%	0	3.0%	2.0%	5.0%	0
		2.0%	3.0%	5.0%	0	2.0%	2.0%	5.0%	0
0	0%	ND	ND	ND	0	ND	ND	ND	1 (TP53)
		ND	ND	ND	0	ND	ND	ND	0

NGS analytical validation : A model tumor cell line (MDA-MB-231) was spiked into healthy donor blood, resulting in final CTC concentrations ranging from 0-12 CTC/mL blood. The cell line has 3 known somatic variants in the BRAF, KRAS, and TP53 genes. The NGS workflow was able to detect each of these variants in 17/18 attempts (Site 1) and 23/24 attempts (Site 2) with only 1 false positive call made across both test groups (N=18 samples).



High Purity Enhancement: Bladder cancer samples (N=15) were processed using the IsoFlux NGS Kit that contains a purity-enhancement column. The mean purity in CTC-positive (>10) samples went from 5% to 15%, a level that is compatible with NGS analysis.

Sample Type	CTC Purity %	Variants Detected via NGS Site 1	Variants Detected via NGS Site 2
Healthy Control	0%	N/A	N/A
Bladder - Neoadjuvant	19%	PDGFRA (1%)	PDGFRA (1%)
Bladder - Neoadjuvant	10%	MET (1.5%)	PIK3CA (1.3%)
Bladder -Metastatic	25%	JAK2 (12%)	JAK2 (12%)
Bladder -Metastatic	11%	None	None
Positive Control #1 (MDA-MB-231 spike in)	16%	BRAF (6%) KRAS (6%) TP53 (11%)	BRAF (6%) KRAS (6%) TP53 (11%)
Bladder - Neoadjuvant	12%	ATM (1%) SMARCB1 (1%)	Not tested
Bladder -Metastatic	15%	NOTCH1 (1%)	
Bladder - Neoadjuvant	8%	EGFR (6%) FBXW7 (1%)	
Bladder -Metastatic	ND	None	
Positive Control #2 (MDA-MB-231 spike in)	16%	BRAF (6%) KRAS (4%) TP53 (11%)	

NGS on clinical samples: Bladder cancer samples (N=4 neoadjuvant, N=4 metastatic) were enriched for CTCs, lysed, amplified, and sent through the NGS analysis workflow. Somatic variants were detected in 6/8 (75%) of samples.

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CONCLUSIONS

The IsoFlux System employs multiple capture antibodies to recover CTCs from multiple indications using a routine blood draw

IsoFlux currently being used in numerous translational studies to monitor patients and profile tumor cells using NGS analysis

NGS workflow has been developed and validated to produce high-confidence somatic variants