Mutational analysis of circulating tumor cells in breast cancer patients by targeted clonal sequencing

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Background

- The genomic signature of circulating tumor cells (CTCs) may serve as a surrogate marker to accurately describe the metastatic tumor of interest
- Current CTC enrichment remains highly heterogeneous with a large amount of white blood cells contamination
- Given its ultra depth of coverage, the NextGen sequencer may allow detection of rare somatic mutations in enriched CTC samples

Methods: CTC Enrichment

- Cross-sectional study to collect CTCs in patients with metastatic breast cancer at Columbia University Medical Center since September 2011 (ongoing)
- CTCs enriched by the following:
  - Microfluidic device
  - Antibodies against both epithelial and mesenchymal markers

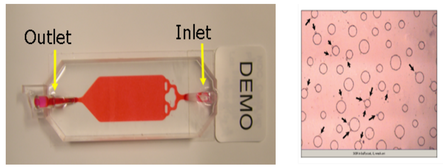


Figure 1: Post-based Microfluidic Channel

Methods: Mutation Analysis

- Emulsion-based multiplex-PCR targeted for various cancer genes including PIK3CA performed on genomic DNA extracted from CTC samples
- Semiconductor-based deep sequencing completed
- Two types of negative controls used and sequenced with the same approach
  - Peripheral blood mononuclear cells (PBMC) from a healthy female volunteer
  - Personalized flow-through PBMC from the enrichment device
- Statistical Model:
  - Reads error rate for each putative mutation site based on the following:
    - Sequence context
      - 5' homopolymer size, local GC content, and position of the base on each read
    - Quality scores
  - Dirichlet-multinomial distribution to model the errors
  - Mutation is confirmed if the null model is rejected (i.e. posterior error rate ≤ recalibrated rate)

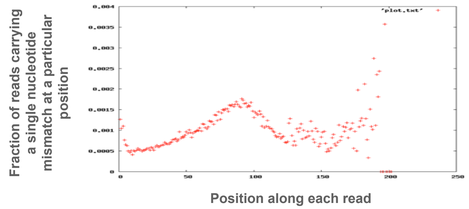


Figure 2: Mismatch error estimate for Ion Torrent run on E coli reference genome. All mismatches viewed as errors. Overall, the single nucleotide error rate is less than 0.2%

Results

- Of the 25 samples, CTCs were enriched in 12 patients
- Multiplex targeted sequencing performed on DNA from six enriched CTC patient samples (purity > 0.3%)

Table 1: Patient Recruitment (n=25/40)

Patient Characteristics	Number
N	25
Age	56 [30-79]
ER+/PR + (Primary Tumors)	22/25
HER2+ (Primary Tumors)	5/25
# of prior lines of metastatic chemotherapy	Average=3, Range= [1-8]

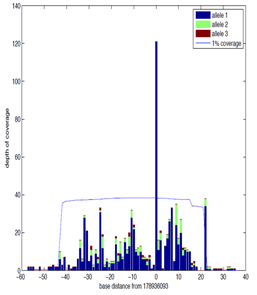
Table 2: CTC enrichment results

CTC Enrichment	Number
CTC ≥ 1	12
Count	
• Mean • Median	170 [1, 1296] 16
Purity	
• Mean • Median	1.94% [0.01-10%] 0.40%

Table 3: CTC *PIK3CA* E545D mutation analysis

Patient (Pt)	CK+/ CD 45- (CTCs)	CK-/ CD 45+ (WBCs)	Variance Freq	P-value	Cover-age
1	1063	17522	3.12	2.00 e-07	3402
2	563	4894	5.86	1.26 e-07	871
3	76	2109	NIL	N/A	506
4	32	5600	NIL	N/A	831
5	12	3321	17.15	1.00 e-10	799
6	17	1938	NIL	N/A	970
Negative Control #1 •Healthy Subject's WBC	NIL	N/A	NIL	N/A	1771
Negative Control #2 •Matched Flow-through WBC:pt 1, 2	NIL	N/A	NIL	N/A	1736

Figure 3. Read error analysis: # of mismatch in the region of PIK3CA E545D mutation



Results (cont' d)

Table 4: Additional Mutations in Exons Regions (patients 1 and 2)

Gene	Missense Mutation	P-value
PIK3CA	L540F (novel)	1.3 e-04
PIK3CA	Q1033K(novel)	1.3 e-04
STK11	F354L	2.0 e-07
NRAS	Q61R	2.0 e-07

Conclusions

- It is feasible to use deep sequencing to detect rare somatic mutations in highly heterogeneous enriched CTC samples
- We are comparing the mutational profile between CTCs and primary/metastatic tumors (ongoing)
- Novel mutations associated with tumorigenesis and/or metastasis may be explored using this method
- We intend to validate clonal mutational analysis of CTCs as a predictive blood-based biomarker in subsequent trials

References Cited

1. Wheeler, D.A., et al., *The complete genome of an individual by massively parallel DNA sequencing.* Nature, 2008. **452**(7189): p. 872-6.
2. Rothberg, J.M., et al., *An integrated semiconductor device enabling non-optical genome sequencing.* Nature, 2011. **475**(7356): p. 348-52.
3. Pecot, C.V., et al., *A novel platform for detection of CK+ and CK- CTCs.* Cancer Discov, 2011. **1**(7): p. 580-586.
4. Mikolajczyk, S.D., et al., *Detection of EpCAM-Negative and Cytokeratin-Negative Circulating Tumor Cells in Peripheral Blood.* J Oncol, 2011. **2011**: p. 252361.

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