CTC vs. biopsy tissue sequencing: a concordance analysis of genomic copy number profile from mCRPC patients (pts)

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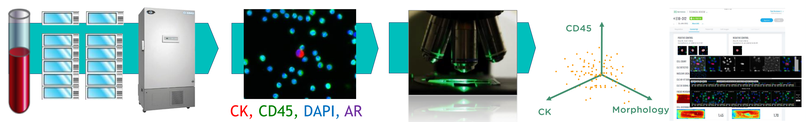
BACKGROUND

- MSK-IMPACT™ (Integrated Mutation Profiling of Actionable Cancer Targets), is a high throughput, targeted-DNA-sequencing panel for somatic mutations created by the Department of Pathology at Memorial Sloan Kettering Cancer Center (MSK) that is FDA approved for tumor tissue profiling to guide treatment selection.
- Recognizing access to tumor tissue for profiling in many cancers is difficult and may harbor inter- & intra-lesional heterogeneity, we evaluated
  1) The ability to obtain tumor material for profiling from patients with metastatic castration resistant prostate cancer who underwent a biopsy of a metastatic lesion and who had a blood sample drawn to profile CTC.
  2) the concordance of sequencing single CTCs vs. paired biopsy analyzed by MSK-IMPACT, to assess differences in the alterations identified, clonality, and their relationship to outcomes.

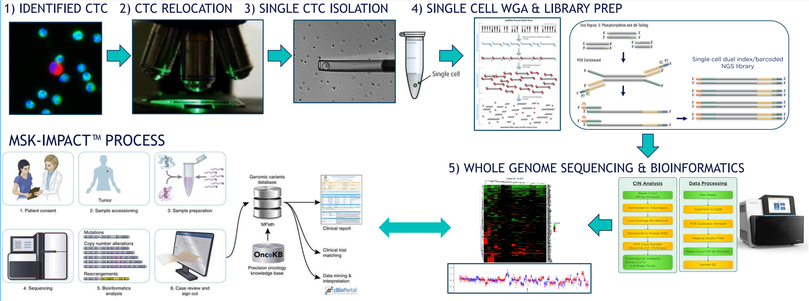
METHODS

Epic Sciences CTC Detection



1) Nucleated cells from patient blood samples are deposited onto glass slides; 2) Slides are stained; 3) Scanned automatically to detect DAPI, CK, CD45, & AR; 4) CTC identification based on (DAPI+; CK+; CD45-) phenotype using a multi-parametric digital pathology algorithm.

Genomics Processing & Methodology



Single CTC Sequencing:

1-3) Identified CTCs were relocated and captured individually. 4-5) Each recovered cell was lysed, whole genome amplified (WGA), shotgun dual index NGS-library prepared and low pass whole genome sequenced using Illumina NextSeq 500. CNV analysis was performed as previously described (Greene et al., PLoS 2016).

MSK-IMPACT™ Sequencing:

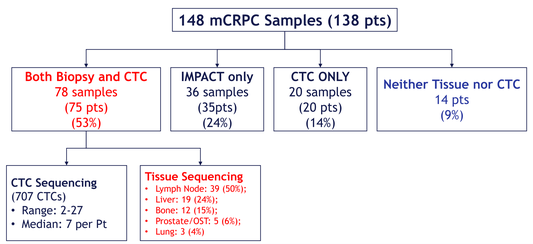
DNA derived from matched fresh biopsy was sequenced as previously described by the MSK-IMPACT tumor sequencing. For purposes of comparison, CNVs were called from across the panel using the same CNV pipeline used for single cells.

Patient Demographics

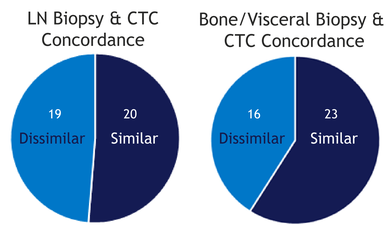
Patient Characteristics	
Total Samples	148 (139 Unique Pts)
1st Line	41 (28%)
2nd Line	28 (19%)
3rd Line	13 (9%)
4th+ Line	66 (45%)
Pre-therapy Clinical Measures: Median (range)	
Age (years)	67 (47, 86)
Albumin (g/dL)	4.1 (2.6, 4.8)
Hemoglobin (g/dL)	12.3 (7.9, 14.9)
LDH (U/L)	233 (126, 873)
PSA (ng/mL)	28.2 (0, 16275)
Alkaline Phosphatase (U/L)	113 (42, 725)
Therapeutic Regiments	
Docetaxel or Cabazitaxel	53 (36%)
Abiraterone or Enzalutamide	47 (32%)
Cisplatin or Carboplatin	6 (4%)
Experimental Agent	42 (28%)
Site of Metastasis	
Lymph Node	68 (46%)
Bone	43 (29%)
Liver	23 (16%)
Prostate/OST	9 (6%)
Lung	5 (3%)

Abbreviations: tx, therapy; LDH, lactate dehydrogenase; PSA: prostate-specific antigen

Success Rates of Obtaining Tumor Material by Biopsy and CTCs



OVERALL CONCORDANCE BETWEEN CTC AND MATCHED TISSUE

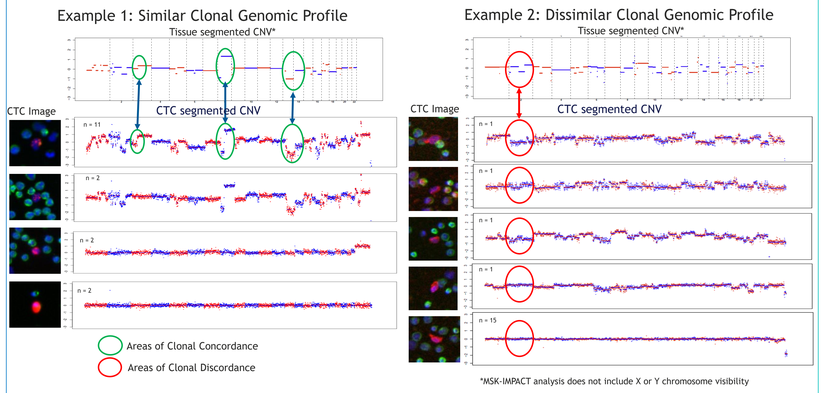


ALL	LN	Bone Marrow or other visceral sites	Total
Similar	20	23	43
Dissimilar	19	16	35

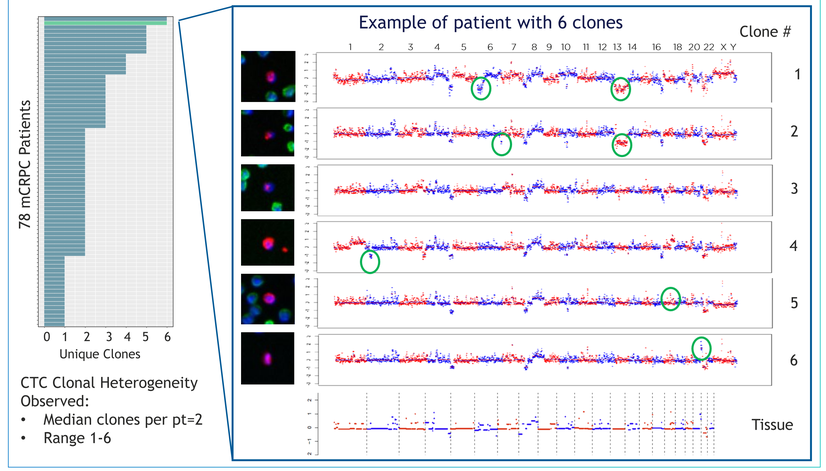
Fisher test, p value = 0.65

1. Rates calculated for samples with a minimum of 2 CTCs sequenced
2. Concordance was determined by the similarity (>60%) of two genome profiles and if they share the same truncal alterations. Data was reviewed by three genomics bioinformatician and scientists.

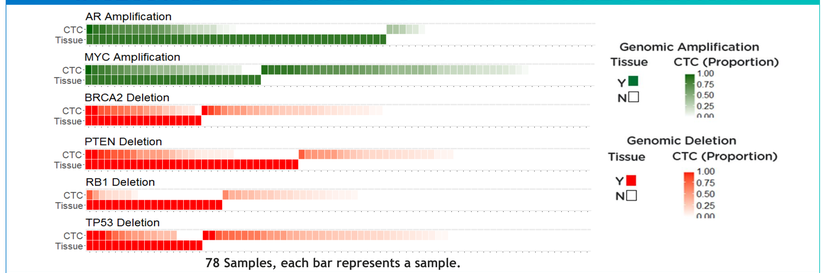
MULTICLONALITY IDENTIFIED IN INDIVIDUAL CTCS NOT IN BIOPSY



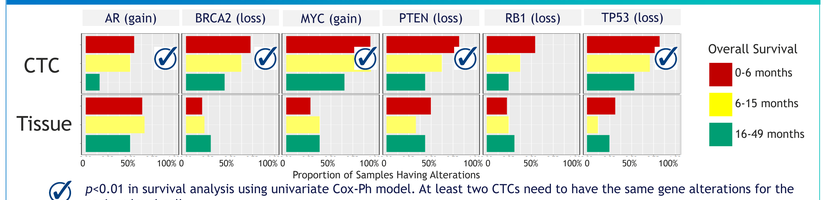
CTC AND MATCHED TISSUE DEMONSTRATE CONCORDANT AND DISCORDANT GENOMIC PROFILES



CTC IDENTIFIED A HIGHER OCCURRENCE AND SUBCLONAL CNVS, INCLUDING POTENTIALLY ACTIONABLE BRCA2 DELETIONS

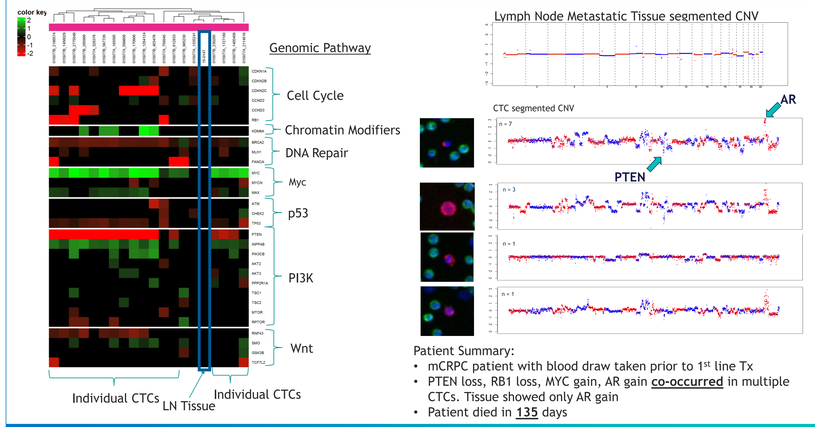


CANCER DRIVER GENE ALTERATIONS DETECTED IN CTCS IMPROVE PROGNOSTICATION VS TISSUE



✓ $p<0.01$ in survival analysis using univariate Cox-Ph model. At least two CTCs need to have the same gene alterations for the patient level call .

CASE STUDY: CTC SEQUENCING CAN PROVIDE ACTIONABLE INFORMATION WHEN TISSUE IS NOT INFORMATIVE



Patient Summary:
- mCRPC patient with blood draw taken prior to 1st line Tx
- PTEN loss, RB1 loss, MYC gain, AR gain **co-occurred** in multiple CTCs. Tissue showed only AR gain
- Patient died in **135** days

CONCLUSIONS

- Overall, tumor material for profiling was obtained in 91% cases, 77% by biopsy, 67% by CTC, and 53% by both.
- Single CTC sequencing is concordant to metastatic tissue in about ~50% pts, and unique CTC clones highlight the prevalence of sub-clonal disease in mCRPC patients under-sampled by tissue biopsy.
- CTC genomic profiling provides a clinical alternative to characterize patient's disease in real time when tumor biopsy material is insufficient/inadequate.
- We don't know which profile is most predictive of treatment success if an actionable molecular alteration is identified.
- Known genomic alterations of progressive mCRPC are frequently observed in CTCs from patients with short OS.

FUNDING AND ACKNOWLEDGEMENTS: Equal distributions of funds from NIH/NCI P50-CA92629 SPORE in Prostate Cancer, NIH/NCI Cancer Center Support Grant P30-CA008748, NIH grant R01-CA207220, Department of Defense Prostate Cancer Research Program (PC121111 and PC131984), Prostate Cancer Foundation Challenge Award, and David H. Koch Fund for Prostate Cancer Research were used to support the design and conduct of the study at Memorial Sloan Kettering Cancer Center.