Clonal Concordance and Genomic Heterogeneity in Single CTC Copy Number Alterations vs. Paired IMPACT Metastatic Tissue Sequencing from mCRPC Patient Samples

Howard I. Scher[1], Angel Rodriguez[2], Jerry Lee[2], Ramsay Sutton[2], Ryon P. Graf[2], Nicole Schreiber[1], Melanie Hullings[1], Yipeng Wang[2], Mark Landers[2], David Solit[1], Michael Berger[1], Nikolaus Schultz[1], Ryan Dittamore[2]

[1] Sidney Kimmel Center for Prostate and Urologic Cancers, Memorial Sloan Kettering Cancer Center, New York, NY [2] Epic Sciences, Inc. San Diego, CA

Epic Sciences

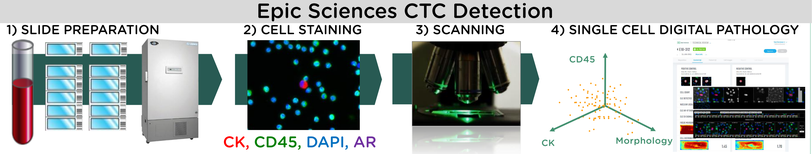
Memorial Sloan Kettering Cancer Center

Background

High CTC phenotypic heterogeneity is associated with non-response to ARSi but not taxane chemotherapy assessed using a non-invasive rapid blood test. The MSK-IMPACT™ NGS assay is FDA approved for tumor tissue profiling to guide treatment selection. The frequency of directly actionable alterations in prostate cancer (PC) is ~35%. Recognizing many cancers harbor intra-, inter-, and intercellular heterogeneity we sought to evaluate concordance of sequencing single CTCs vs. paired biopsy analyzed by MSK-IMPACT, to assess CTC clonality in circulation vs. tumor, the relationship to CTC phenotypic heterogeneity and response.

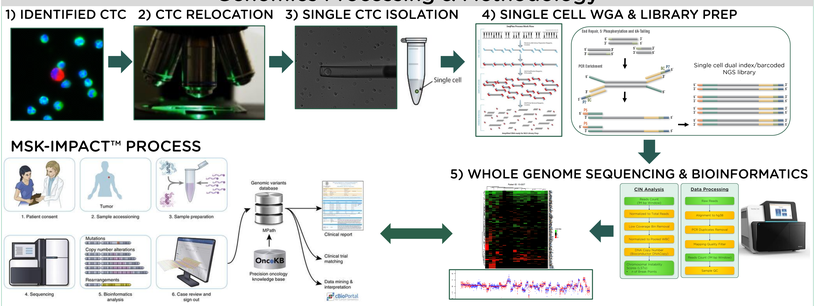
Methods

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 CTCs were individually isolated. 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 perform as previously described.

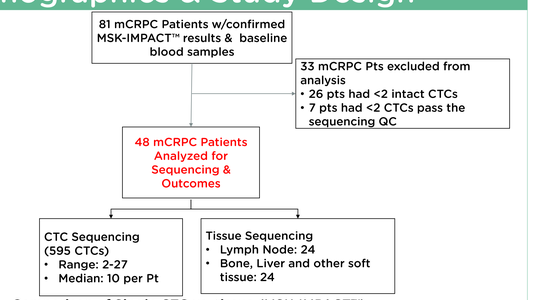
MSK-IMPACT™ Sequencing:

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

Patient Demographics & Study Design

Patient Characteristics at time of Blood Draw and Metastatic Biopsy	
Total Samples	48
1st Line	18 (38%)
2nd Line	8 (17%)
3rd Line	9 (19%)
4th+ Line	13 (27%)
Median Overall Survival (months)	11.0 (6.31 – 20.7)
Pretherapy Clinical Measures: Median (range)	
Age (years)	70 (49 – 85)
Albumin (g/dL)	4.1 (3.2 – 4.4)
Hemoglobin (g/dL)	11.7 (8.2 – 14.5)
LDH (U/L)	253 (158 – 838)
PSA (ng/mL)	29.5 (0.07 – 6905)
Alkaline Phosphatase (U/L)	122 (34 – 1574)
Therapeutic Regiments	
Docetaxel or Cabazitaxel	10 (21%)
Abiraterone, Enzalutamide or Apalutamide	13 (27%)
Experimental Agent	25 (52%)
Site of Metastasis	
Lymph Node	24 (50%)
Bone	9 (19%)
Liver	9 (19%)
Other	6 (12%)

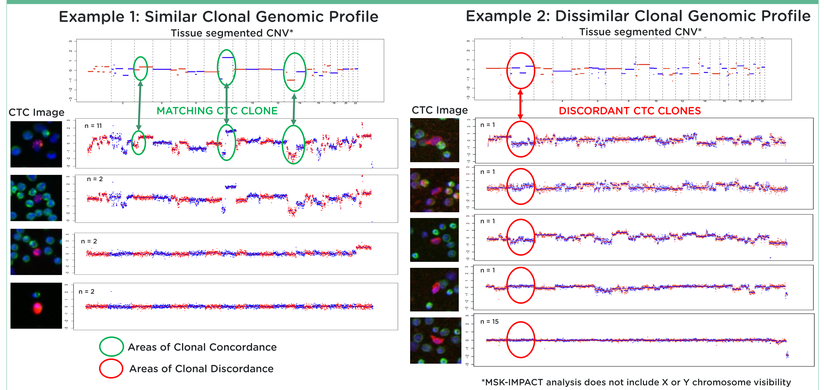
Abbreviations: tx, therapy; LDH, lactate dehydrogenase; PSA: prostate-specific antigen



Comparison of Single CTC vs. tissue (MSK-IMPACT™)

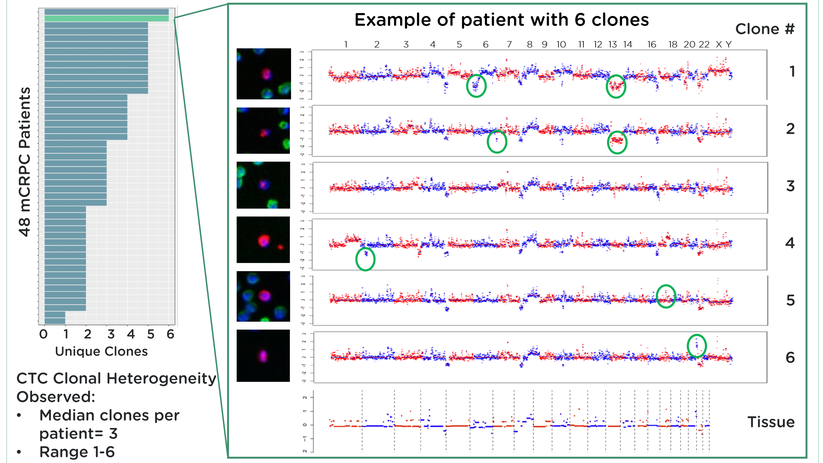
Blinded clonal assessment of CTCs were determined by a trained bioinformatician, blinded of MSK-IMPACT results, location of biopsy, or patient outcome data. Patients were excluded in which less than two sequenced CTCs were observed, due to required observation to ensure CTC comparison

CTC and Matched Tissue Demonstrate Concordant and Discordant Genomic Profiles



*MSK-IMPACT analysis does not include X or Y chromosome visibility

Prevalence of Multiple Unique Genomic Clones Observed in CTCs

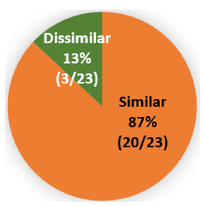


CTC Clonal Heterogeneity Observed:

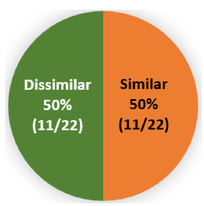
- Median clones per patient= 3
- Range 1-6

High Clonal Concordance in Bone/Visceral; Low Concordance with LN Metastatic

Bone/Visceral Biopsy & CTC Concordance



LN Biopsy & CTC Concordance

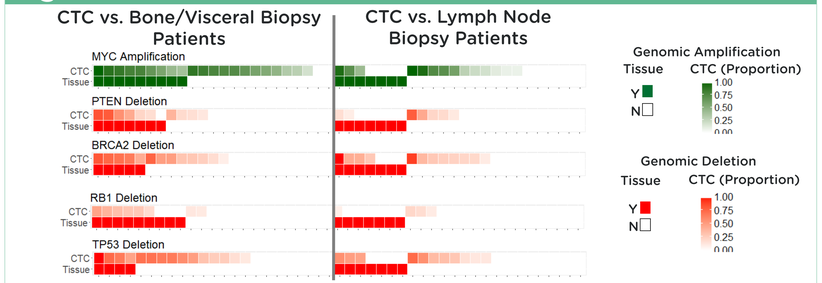


	Similar	Dissimilar
Lymph Node	11	11
Bone/Visceral	20	3

p=0.011 by Fisher's exact test.

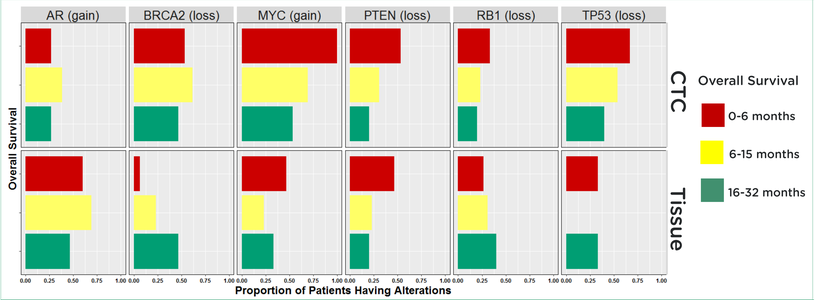
- Three patients (3/48) were unevaluable.

High Occurrence of Resistant Genomics Identified in CTCs

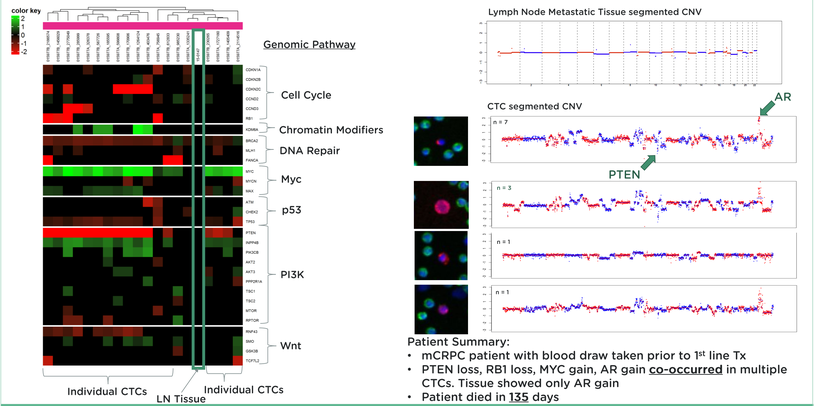


Known oncogenes and tumor suppressors of mCRPC progression were analyzed in context of the sites of metastatic biopsy and genomic call

Genomic Alterations Identified in CTCs & Tissue Associate with Survival



Case Study: CTC Genomic Profile in Aggressive Disease



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

- Single CTC sequencing is often concordant to metastatic tissue, but unique CTC clones highlight the prevalence of sub-clonal disease in mCRPC patients under-sampled by tissue biopsy.
- Lymph node biopsy may under-represent the cancer cells circulating in the blood, leading to lower utility of genomic calls in these patients.
- Known genomic alterations of progressive mCRPC are frequently observed in CTCs from patients with short OS.

Reference: Chromosomal Instability Estimation Based on Next Generation Sequencing and Single Cell Genome Wide Copy Number Variation Analysis. Greene SB, Dago AE, et al., PLoS One. 2016 Nov 16;11(11):e0165089.

Funding: 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.