Non-invasive profiling of advanced prostate cancer via multi-parametric liquid biopsy and radiomics

USC Norris Comprehensive Cancer Center
Part of the Keck School of Medicine of USC

Gareth Morrison[1], Jonathan Buckley[2], D. Gigi Ostrow[2], Bino Varghese[3], Nolan Ericson[4], Steven Cen[3], Alexander Cunha[1], Yi-Tsung Lu[1], Tad George[4], Jeffrey Smith[5], David Quinn[1], Vinay Duddalwar[3], Timothy Triche[2], Amir Goldkorn[1]

[1] Department of Medicine, Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA; [2] Department of Pathology and Laboratory Medicine, CHLA, Los Angeles, CA; [3] Department of Radiology, Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA, [4] RareCyte, Seattle, WA, [5] Thermo Fisher Scientific, Calsbad, CA

ABSTRACT

Introduction: Biomarkers of treatment response in advanced prostate cancer (PC) are urgently needed. Liquid biopsy profiling and radiomic imaging enable non-invasive, repeatable tumor profiling, and combining these modalities could yield powerful new predictive tools. We tested the feasibility of concurrent, multi-parametric molecular analysis of circulating tumor cells (CTCs) and matched plasma cell-free DNA (cfDNA), combined with radiomic analysis of CT scans from the same patients.

Methods: Under IRB-approved informed consent, blood was collected from 23 patients with metastatic castrate resistant PC (mCRPC). CTCs were enumerated using the RareCyte and CellSearch platforms, and individual single CTCs and white blood cells (WBCs) were retrieved using RareCyte's integrated robotic micropipette. DNA was extracted from single cells (scDNA), as well as from matched cfDNA and buffy coats (bcDNA), then sequenced using Ampliseq HD Pan-cancer panel. Concurrent radiomic analysis was performed on bone metastatic lesions identified on CT scans from the same patients, and manually segmented regions of interest were queried for a variety of texture metrics.

Results: Of 19 patients with both CellSearch and RareCyte enumeration, CTCs were detected in 12 by CellSearch (63%; median: 3/7.5 mL; range 1-343/ 7.5 mL) and in 14 by RareCyte (74%; median: 1/7.5 mL; range 1-363/7.5 mL). Using matched bcDNA as a gold standard, sensitivity and positive predictive value of germline SNP detection were 74% and 93% for scDNA, and 98% and 99% for cfDNA. A total of 48 and 18 somatic alterations were identified across the cohort in CTCs and cfDNA, respectively, including mutations in AR, TP53, CCND3, FGFR1, ALK, and ROS1. Of 14 patients whose single CTCs were recovered by RareCyte, 12 (86%) had detectable somatic mutations in scDNA, and 7 (50%) had detectable somatic mutations in matched cfDNA. While some mutations were concordant between matched scDNA and cfDNA, most were distinct. Radiomic entropy in CT scans was associated with CellSearch CTC-counts (AUC of 0.74) using the FDA-cleared prognostic threshold of <5 vs. ≥5 CTCs/7.5ml.

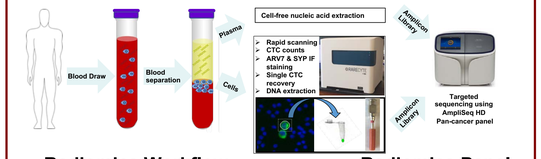
Conclusion: We successfully piloted an integrated, multi-parametric analysis of matched single CTCs, WBCs, plasma cfDNA, and buffy coats, along with matched radiomic analysis of CT scans in patients with mCRPC. Germline variants were detected more readily in cfDNA, whereas somatic mutations in commonly altered genes were detected more frequently in CTCs. CellSearch CTC count, an FDA cleared prognostic biomarker, correlated with radiomic entropy, the first such association to our knowledge between liquid biopsy and radiographic readouts. The results demonstrate the feasibility of this approach and its potential to generate comprehensive molecular data and new biomarker profiles. Prospective validation in a large PC cohort is underway.

BACKGROUND

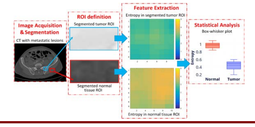
- **Despite new life extending therapies, prostate cancer is still the most common and second most lethal malignancy in U.S men.**
- **Better biomarkers are needed that can predict and monitor a patient's treatment response; measuring these biomarkers must be repeatable, affordable and minimally invasive.**
- **Liquid biopsy profiling and quantitative radiomic analysis are two approaches that can be synergized to identify signatures of disease response and resistance.**
- **Here we test the feasibility of parallel liquid biopsy profiling and radiomic analysis from matched mCRPC patients, whilst exploring associations between both modalities.**

METHODS

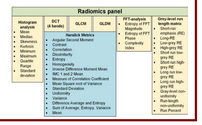
Multi-Parametric Liquid Biopsy Workflow



Radiomics Workflow



Radiomics Panel



Liquid Biopsy Workflows

•Peripheral blood was collected in CellSave, RareCyte and DNA Streck BCTs.

•CTCs were enumerated (CellSearch® and RareCyte Systems)

•ARv7 and SYP IF staining (Leica Bond RXm / RareCyte)

•Single CTCs and control WBCs recovered using RareCyte's CytePicker® Retrieval Module.

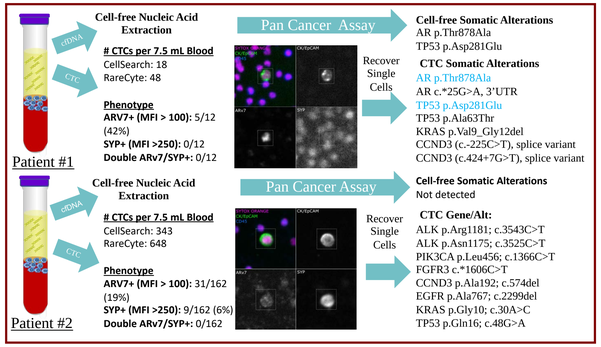
•Recovered scDNA, in parallel with matched plasma cfDNA was used to prepare NGS libraries using Ampliseq HD Pan-cancer panel and sequenced.

Radiomics Worklow

•Radiomic analysis performed on bone metastatic lesions identified on CT scans

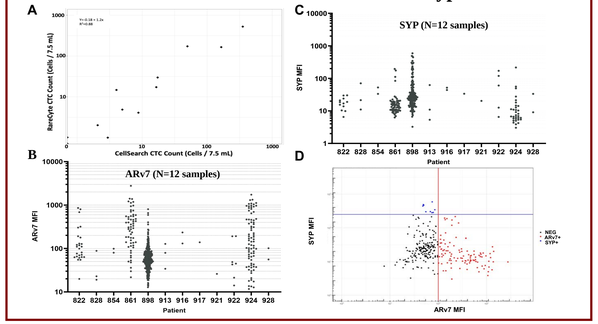
•Radiomics-based texture analysis (see Radiomics Panel) was performed on the segmented ROIs

RESULTS



Multi-parametric liquid biopsy analyses of individual patients. Patient #1 (top) had identifiable CTCs across both detection platforms, with the fraction of CTCs positive for ARv7 and SYP shown (2 out of 8 slides analyzed). Somatic alterations were identified in both cfDNA and CTCs (total of 3 sequenced). Patient #2 (bottom) had identifiable CTCs across both platforms, with the fraction of CTCs positive for ARv7 and SYP shown (2 out of 8 slides analyzed). Somatic alterations were identified only in CTCs (total of 3 sequenced). Blue indicates somatic alterations identified in both cfDNA and CTC DNA.

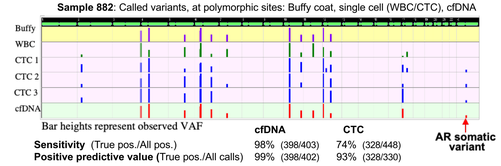
CTC Counts and Cellular Phenotype



Summary of CTC enumeration and cellular phenotyping from 23 mCRPC patients. (A) CTC counts displayed as a comparison between Cell Search and RareCyte counts. (B) ARv7 MFI distribution for 517 CTCs from 12 prostate cancer patients. (C) SYP MFI distribution for 517 CTCs from 12 prostate cancer patients. (D) SYP vs ARv7 MFI distribution for 517 CTCs from 12 prostate cancer patients. NEG stands for identified CTCs negative for both ARv7 and SYP.

RESULTS (cont)

A Germline Variants



	cfDNA	CTC
Sensitivity (True pos./All pos.)	98% (398/403)	74% (328/448)
Positive predictive value (True pos./All calls)	99% (398/402)	93% (328/330)

B Somatic Variants

cf DNA
VAF: 0.3 % - 63%

cf DNA Variants	
Gene	Count
TP53	3
AR	2
PIK3CA	2
FGFR2	1
CDK4	1
BRAF	1
PTEN	1
MAP2K2	1
CHEK2	1
IDH1	1

Both cf DNA & CTC
(in same patient)

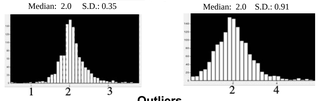
Gene	CTCs
AR	4 of 4
TP53	3 of 4
PTEN	2 of 4
IDH1	1 of 4

CTC
VAF > 20%

CTC Variants		CDK6	1
Gene	Count	FGFR1	1
TP53	7	FGFR2	1
ALK	4	FGFR3	1
CCND3	3	KRAS	1
EGFR3	3	MAP2K2	1
AR	2	MET	1
KRAS	2	ROS1	1
PTEN	2	ERBD2	1

Most seen only in single CTCs

C Copy Number (CN)



Outliers

cf DNA CN variants (> 3.5)	
Gene	CN
AR†	4.9, 7.1, 5.0, 30.4
FGFR1	4.2, 4.4
FGFR3	3.6
ESR1	7.8
GNAQ	7.7
CCND1	3.6
GNA11	4.2

CTC CN variants (>4.5 & N > 1)		
Gene	N	CN
AR†	14	4.5 – 53.2
SYN1†	4	4.5, 4.8, 5.0, 5.2
FGFR1	3	5.1, 7.2, 8.3
HRAS	2	5.6, 6.0
NTRK1	2	4.5, 5.5
FGFR3	2	4.7, 8.6
TP53	2	5.4, 12.1
CCND2	2	4.5, 5.0

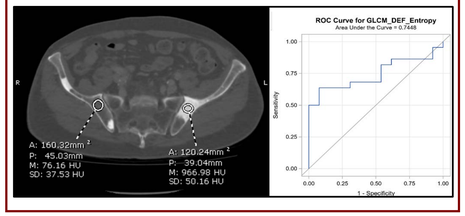
AR† Calls (cf vs. CTC)

	cfDNA		CTCs			
Sample	CN	N	CN			
920	30.4	4	40.1	39.7	16.5	**3.3**
861	14.2	4	15.4	15.2	14.4	14.0
822	7.1	4	7.5	7.5	5.8	5.3
921	6.5	1	53.2			
913	5.0	2	7.5	4.3		
924	5.9	2	5.4	**2.3**		

†X-chromosome genes (AR & SYN1) are scaled to a baseline of CN = 2, for comparability

Summary of liquid biopsy (CTC and cfDNA) genomic profiling results of samples from 23 mCRPC patients. (A) Variant calling sensitivity and positive predictive value estimated using germline variant detection in cfDNA and CTCs. (B) Detection of somatic variants in cfDNA and CTC across patient cohort (depicts updated data showing identified variants based on an updated VAF cutoff). (C) Distribution of gene-level copy number (CN) estimates in cfDNA and CTCs, with copy number variants tabulated separately. Androgen Receptor CN estimates show remarkable consistency, both between cfDNA and CTC analysis, and between CTCs (bottom table).

Radiomic Entropy vs CellSearch Counts



Radiomic analysis. Representative CT scan with the bone metastatic lesion (left) and normal tissue (right) ROIs indicated. (Right) Radiomic-based texture metric, entropy extracted from GLCM maps was associated with CellSearch CTC Counts using <5 vs. ≥5 CTCs/7.5mL cutoff with an AUC of 0.74.

ACKNOWLEDGEMENT

Funding: 5P30CA014089-42 . Partial support provided by Thermo Fisher Scientific and RareCyte for assays conducted using their respective platforms.

CONCLUSIONS

- In this pilot study, concurrent multi-parametric liquid biopsy profiling and quantitative radiomic analysis was feasible.
- Somatic alterations (SSNVs & CN) were identified in both CTCs and cfDNA, with both shared and importantly unique alterations identified providing a more comprehensive genomic profile.
- To our knowledge, this is the first association between CTC counts and CT scan derived radiomic metric in metastatic prostate cancer.

FUTURE DIRECTIONS

- This combined liquid biopsy and radiomic workflow is being integrated, to prospectively analyze a larger patient cohort.
- Development and validation of a new targeted sequencing panel specific for prostate cancer is ongoing.