Integrated Single Cell Phenogenomic Subtyping of CTCs Identify Inter-Cellular Tumor Heterogeneity (het) and Multiple Resistance Mechanisms in mCRPC Patients (pts)

Howard I. Scher [1], Ryon Graf [2], Adam Jendrisak [2], Nicole A. Schreiber [1], Brigit McLaughlin [1], Stephanie Green [2], Angel Rodriguez [2], Martin Fleisher [1], Jerry Lee [2], James Kelvin[2], Mahipal Suraneni [2], Yipeng Wang [2], Mark Landers [2], Ryan Dittamore [2]

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

Background

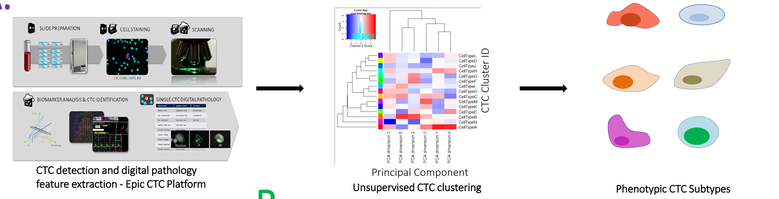
Intra-tumor and inter-cellular heterogeneity is highly prevalent in progressing mCRPC pts and has been associated with resistance to ARSi Tx.[1] While genomic profiling of metastatic tumors may identify pts with actionable targets, the response to novel Tx outside of ARSi, taxanes and DDR directed drugs are infrequent and not durable. Basket trials have also had limited impact for mCRPC pts. We sought to better understand tumor heterogeneity at the single cell level for the purposes of:

- Better defining the sensitivity of the individual circulating tumor cell (CTC) subtypes to ARSi Tx & taxane-based chemotherapy
- Better understanding tumor evolution and better characterization of subclonal tumor types, mechanisms of resistance & clustering of genetic changes
- Developing more effective biomarker directed treatment approaches for heterogeneous tumors

CTC Based Phenogenomic Analysis Using Digital Pathology and NGS Reveals CTC SubTypes Associated with Clinical Outcomes

A.

Methodology



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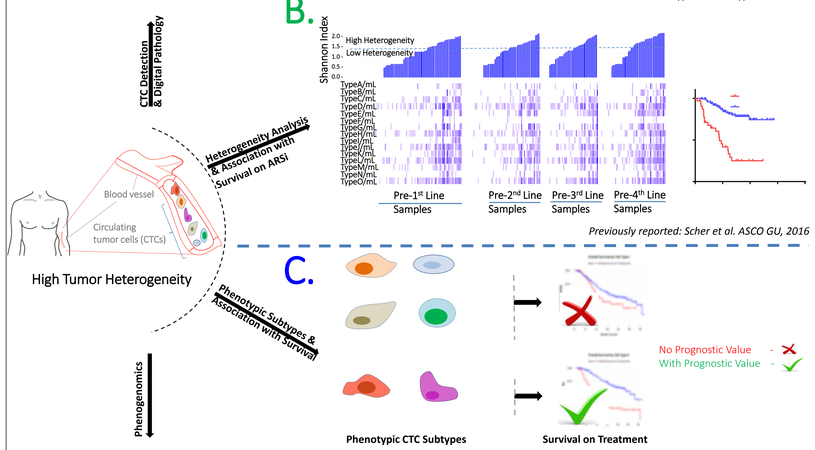

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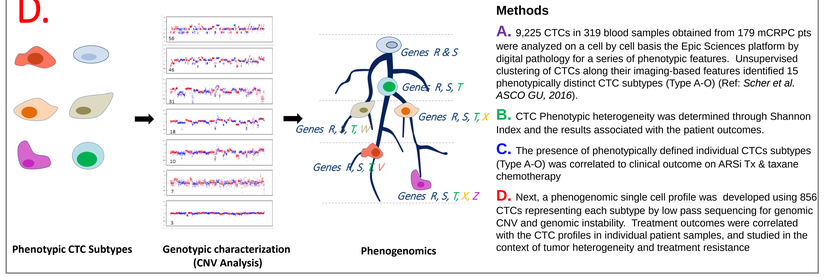


Methods

A. 9,225 CTCs in 319 blood samples obtained from 179 mCRPC pts were analyzed on a cell by cell basis the Epic Sciences platform by digital pathology for a series of phenotypic features. Unsupervised clustering of CTCs along their imaging-based features identified 15 phenotypically distinct CTC subtypes (Type A-O) (Ref: *Scher et al. ASCO GU, 2016*).

B. CTC Phenotypic heterogeneity was determined through Shannon Index and the results associated with the patient outcomes.

C. The presence of phenotypically defined individual CTCs subtypes (Type A-O) was correlated to clinical outcome on ARSi Tx & taxane chemotherapy

D. Next, a phenogenomic single cell profile was developed using 856 CTCs representing each subtype by low pass sequencing for genomic CNV and genomic instability. Treatment outcomes were correlated with the CTC profiles in individual patient samples, and studied in the context of tumor heterogeneity and treatment resistance

Patient Demographics

Patient Characteristics	
Unique Patients	179
Age, years: median (range)	68 (45-91)
Primary Treatment	
Prostatectomy	84 (47%)
Radiation	34 (19%)
Brachytherapy	7 (4%)
None	54 (30%)
Sample Characteristics	
Total Baseline (pre-therapy) Samples	221
Follow-up (on-therapy) Samples	98
Total Samples	319
Samples with CTCs	264
Prior Hormone Therapies at Baseline [a]	
1-2 lines	82 (37%)
3 lines	50 (23%)
4 or more lines	89 (40%)
Chemotherapy Status at Baseline	
Chemo-naïve	136 (62%)
Chemo-exposed	85 (38%)
Metastatic Therapy Initiated after Baseline	
ARS Inhibitor	150
Taxane	71
Line of Metastatic Therapy at Baseline	
1st line	76
2nd line	56
3rd line	40
≥4th line	49
Metastatic Sites of Disease at Baseline	
Bone Only	70 (32%)
Lymph Node Only [b]	23 (10%)
Bone and Lymph Node [b]	86 (38%)
Bone, Lymph Node, and Visceral [b]	30 (14%)
Laboratory Measures at Baseline	
PSA, ng/mL: median (range)	37.7 (0.1-3728.2)
Hgb, g/dl: median (range)	12 (7-15)
ALK, unit/L: median (range)	110 (25-2170)
LDH, unit/L: median (range) [c]	222.5 (123-1293)
ALB, g/dl: median (range)	4.2 (31.-4.9)
AR N-term CTC Test at Baseline	
Total CTC/mL: median (range)	6.3 (0-991.3)

a - includes GnRH agonists and antagonists, antiandrogens and next-generation hormonal therapies (abiraterone acetate, enzalutamide, and apalutamide)
b - includes patients with other soft tissue disease
c - one sample did not have LDH available

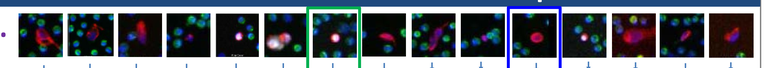
- Standard of care collection from 319 blood samples from 179 mCRPC patients
- Blood draws were collected prior to and while on AR or Taxane therapies
- 221 samples were baseline draws prior to AR or Taxane therapies
- Patients monitored for radiographic progression free survival (rPFS) and overall survival (OS)

Patient Line of Therapy

Total Samples	1st Treatment Decision	2nd Treatment Decision	3rd + Treatment Decision
A or E Baseline Blood Draw (n=150)	No Prior A or E (1st Line) n= 64	Previous A or E (2nd Line) n= 36 Previous T (2nd Line) n= 8	Previous A & E (3rd+ Line) n= 5 Previous AR Tx & T (3rd+ Line) n= 37
T Baseline Blood Draw (n=71)	No Prior A or E (1st Line) n=12	Previous A or E (2nd Line) n= 12	Previous A & E (3rd+ Line) n= 10 Previous AR Tx & T (3rd+ Line) n= 37

Presence of Phenotypic CTC Subtypes Prognosticate Worse Outcomes on ARSi & Taxane Therapies

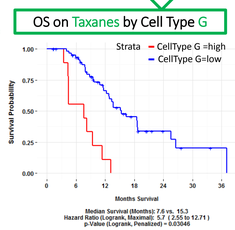
A.



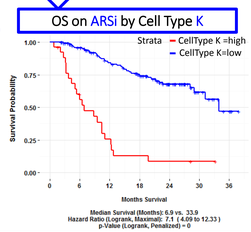
Cell Type	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
p-Value* (on ARSi; *n*=150)	NS*	<0.01	NS	<0.001	<0.00001	NS	<0.001	NS	NS	<0.001	<0.00001	<0.00001	<0.00001	<0.001	<0.00001
p-Value (on Taxanes; *n*=71)	NS	<0.001	NS	<0.05	NS	<0.001	<0.001#	NS	NS	<0.05	<0.01	NS	<0.001	NS	NS

***p-value = continuous association with overall survival on ARSi (logrank)**
***NS = p-value cut-off 0.01; # p-value = 0.00022, lowest p-value among all cell types against Taxanes**

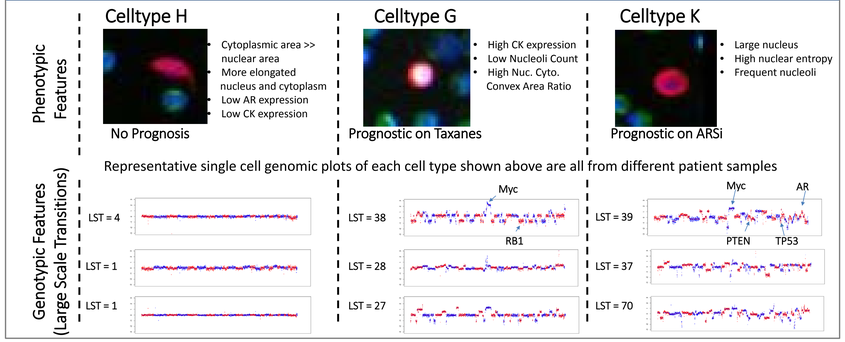
B. OS on Taxanes by Cell Type G



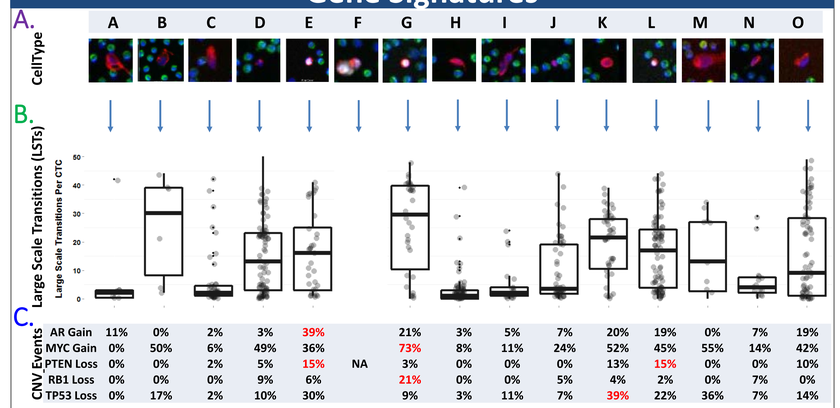
C. OS on ARSi by Cell Type K



PhenoGenomic Profiles of CTC Subtypes

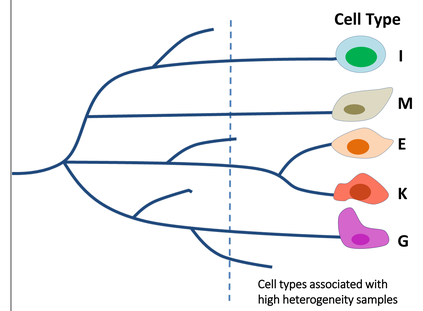


Phenotypic CTC Subtypes are Associated with Distinct Gene Signatures



A. Representative cell images of 15 phenotypic CTC subtypes.
B. Distribution of large scale transitions (LSTs), a surrogate of genomic instability. LST was measured as *n* of chromosomal breaks between adjacent regions of at least 10 Mb.
C. Prevalence of 5 representative cancer gene copy number alterations.

Specific CTC Subtypes are Enriched in Samples with High Heterogeneity Scores



Cell Type	Odds Ratio (HH/LH)	Adjusted p-Value	Enriched CNV Changes
E	5.50	0.0027	AR Amp, MYC Amp, PTEN Loss, TP53 Loss
G	5.22	0.0345	AR Amp, MYC Amp, RB1 Loss
I	12.67	0.0023	MYC Amp, TP53 Loss
K	5.64	0.0001	AR Amp, MYC Amp, PTEN Loss, TP53 Loss
M	15.50	0.0074	MYC Amp, TP53 Loss

- Permutation test (with 100K simulation) was performed to estimate the over-representation of CTC subtypes in high heterogeneity (HH) compared to low heterogeneity (LH) patients
- These five Subtypes were more likely to be detected in high heterogeneity patients, with very low probability of being detected by chance (Bonferroni adjusted p<0.05)
- Overall, these five CTC subtypes also had a higher frequency of copy number alterations than other subtypes (E.g. TP53 loss: 30% vs. 7%; MYC gain: 52% vs. 24%)

Conclusions

- Detecting specific CTC phenotypic subtypes in patients predict the relative risk of failure to ARSi and taxanes, and may inform Rx selection
- CTC subtypes (E, G, K, I, & M) are detected more frequently in high CTC heterogeneity samples (adjusted p-value = 0.05)
- Phenogenomic analysis of CTC subtypes that predict for resistance to ARSi and taxanes and high heterogeneity harbor novel genomic patterns associated with differential prognosis of survival. e.g.: The presence of Cell Type K, enriched for PTEN and p53 loss, is associated with worse prognosis on ARSi and the presence of Cell type G, enriched for RB1 loss showed worse prognosis on Taxanes
- Identifying different CTC subtypes may provide insight into disease evolution, inform treatment decisions for individual patients and guide new drug development. Longitudinal monitoring of phenotypic CTC subtypes in multiple clinical trials is currently underway

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