Baseline Circulating Tumor Cell (CTC) Subtype Predicts Responses to Enzalutamide but Not Abiraterone in Metastatic Castration-Resistant Prostate Cancer (mCRPC) Patients

Howard I. Scher [1,2], Adam Jendrisak [3], Nicole A. Schreiber [1], Brigit McLaughlin [1], Ryon P. Graf [3], Angel Rodriguez [3], Martin Fleisher [1], Jerry Lee [3], James Kelvin [3], Yipeng Wang [3], Mark Landers [3], Ryan Dittamore [3]

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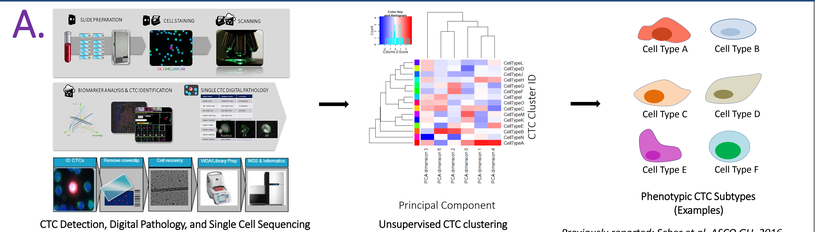
[1] Sidney Kimmel Center for Prostate and Urologic Cancers, Memorial Sloan-Kettering Cancer Center, New York, NY [2] Department of Medicine, Weill Cornell Medical College, New York, NY

[3] Epic Sciences, Inc., San Diego, CA

Background

- In mCRPC patient management, prior response to androgen receptor signaling inhibitors (ARSi), Abiraterone (Abi) or Enzalutamide (Enza) does not predict subsequent response to Enza following Abi or Abi following Enza.
- The detection of nuclear AR-V7 protein in CTCs predicts resistance to both drugs, but does not describe all resistance.
- We previously identified 15 CTC subtypes in mCRPC patients based on unique phenotypic features, each with unique biology and different degree of likelihood of predicting response and resistance to either ARSi or taxanes.
- We explored the CTC subtypes to determine if a particular CTC subtype could predict resistance to either Abi or Enza but not both, and have the potential to guide ARSi therapy selection strategy.

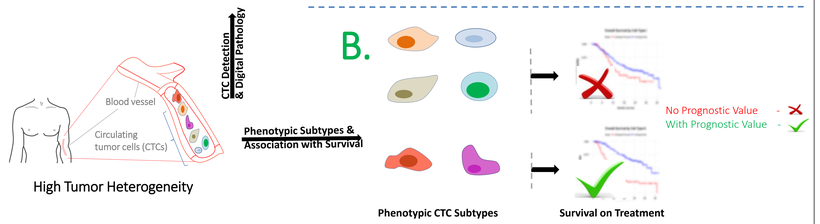
Methods



CTC Detection, Digital Pathology, and Single Cell Sequencing

Unsupervised CTC clustering

Previously reported: Scher et al. ASCO GU, 2016



High Tumor Heterogeneity

A. 9,225 CTCs in 319 blood samples obtained from 179 mCRPC patients 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. The presence of phenotypically defined individual CTCs subtypes (Type A-O) was explored in relation to clinical outcomes on Abi vs. Enza.

Schematic of Epic CTC Platform CTC enumeration, morphology, biomarker, and single cell sequencing (CNV) analyses workflow:

1) Nucleated cells from blood sample placed onto slides and stored in a -80˚C biorepository;
2) Slides stained with cytokeratin (CK), CD45, DAPI, AR N-term
3) Slides scanned and CTC candidates detected by a multi-parametric digital pathology algorithm
4) Human reader confirmation of CTCs & quantitation of biomarker expression
5) 1,324 Single CTCs were lysed, whole genome amplified, shotgun libraries constructed, and whole genome sequenced.
6) Data analyzed for copy number variation analysis (CNV).

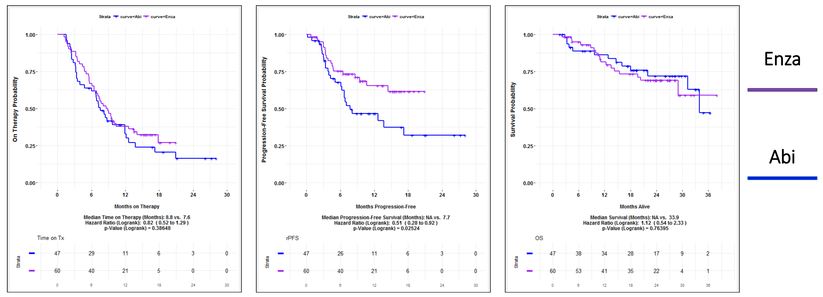
Patient Demographics

Patient Characteristics	
Unique Patients	98
Age, years: median (range)	70 (45-87)
Primary Treatment	
Prostatectomy	47 (48%)
Radiation	21 (21%)
Brachytherapy	3 (3%)
None	27 (28%)
Sample Characteristics	
Total Baseline (pre-therapy) Samples	107
Metastatic Therapy Initiated after Baseline	
Abiraterone	47 (44%)
Enzalutamide	60 (56%)
Line of Metastatic Therapy at Baseline	
1st Line	64 (60%)
2nd Line	43 (40%)
Chemotherapy Status at Baseline	
Chemo-naïve	97 (91%)
Chemo-exposed	10 (9%)

Metastatic Sites of Disease at Baseline	
Bone Only	32 (30%)
Lymph Node Only [a]	20 (19%)
Bone and Lymph Node [a]	45 (42%)
Bone and Visceral +/- Lymph Node [a]	8 (7%)
Other Soft Tissue Only	2 (2%)
Laboratory Measures at Baseline	
PSA, ng/mL: median (range)	20.13 (0.09-2006.14)
Hgb, g/dl: median (range)	12.7 (7.2-15.0)
ALK, unit/L: median (range)	99 (25-2170)
LDH, unit/L: median (range) [b]	207 (123-1293)
ALB, g/dl: median (range)	4.2 (3.3-4.9)
CTC Enumeration at Baseline	
Total CTC/mL: median (range)	2.6 (0 – 2099.6)

[a] - includes patients with other soft tissue disease
[b] - two samples did not have LDH available

Outcomes are similar between Abi or Enza w/o Biomarker



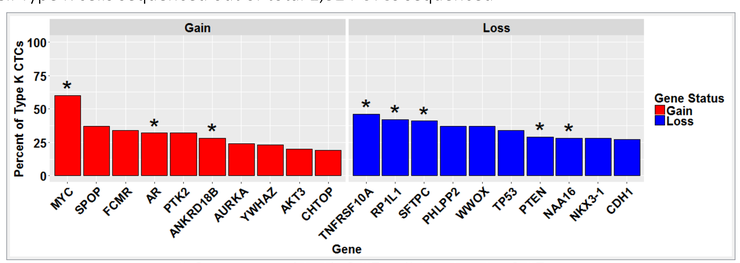
Patients treated with Enza had better rPFS than Abi treated patients, however, time on therapy and overall survival rates were similar.

Phenotypic and Genomic Profiles of Cell Type K

Phenotypic Characteristics
Large nucleus
High nuclear entropy
Frequent nucleoli

Genotypic Characteristics

89 Cell Type K cells sequenced out of total 1,324 CTCs sequenced

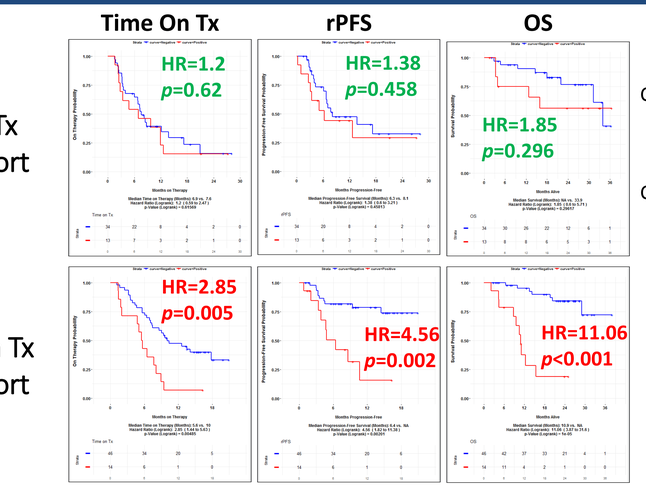


* Genes more enriched in Cell Type K than the other Cell Types

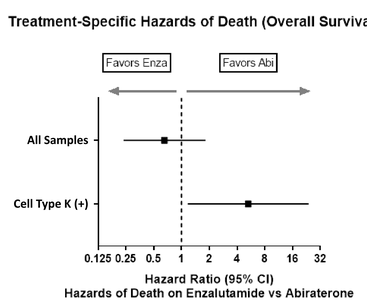
Frequency of Cell Type K in 1st and 2nd Line mCRPC

	Treatment	# Positive	# Negative	Total	% Positive
1st Line	Abi	10	14	24	42%
	Enza	7	33	40	18%
	Abi + Enza	**17**	**47**	**64**	**27%**
2nd Line	Abi	3	20	23	13%
	Enza	7	13	20	35%
	Abi + Enza	**10**	**33**	**43**	**23%**
1st & 2nd Line	Abi	**13**	**34**	**47**	**28%**
	Enza	**14**	**46**	**60**	**23%**

Cell Type K is Prognostic for Poor Outcomes with Enzalutamide, but not Abiraterone



Cell Type K + Patients have Improved OS with Abi over Enza in Multivariate Model



Feature	HR	*p* Value	CI
Enza Therapy	0.6543	0.42	0.2351-1.821
Cell Type K & Enza	5.2792	0.03	1.168-23.861

- Cox proportional model:
  - therapy (Abi vs. Enza)
  - patient Cell Type K status
  - interaction term between therapy and the Cell Type K status.
- Cell Type K status was evaluated as a binary status
  - 1 if patient had 1+ Cell Type K CTC;
  - 0 if Cell Type K CTC count = 0

Conclusions

- The presence of a CTC Subtype (Cell Type K) is found in 25% (27/107) of patients and has a unique phenotypic and genomic profile.
- Cell Type K+ patients have poor outcomes on Enzalutamide in 1st and 2nd line mCRPC therapy but not Abiraterone.
- Exploratory analysis of Cell Type K supports the potential use of the biomarker for stratifying patients on the use of Abiraterone or Enzalutamide.
- Further biologic interrogation of Cell Type K CTCs are ongoing. Clinical validation on the utility of Cell Type K to predict responses to Abi vs. Enza is planned.

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