Molecular Characterization of Circulating Tumor Cells (CTCs) and CTC subpopulations in progressive metastatic Castration Resistant Prostate Cancer (mCRPC)

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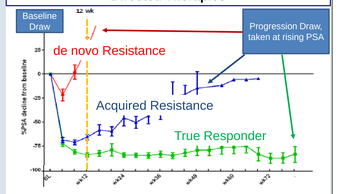
Background

Numerous resistance mechanisms have been postulated in progressive mCRPC. Determining the presence of putative predictive biomarkers in patients requires a real-time tumor assessment because the biology changes under the influence of the specific therapy(ies) that a patient (Pt) has received. We examined CTC and CTC subpopulation incidence and molecular characterization of tumors from Pts with progressive mCRPC to assess if a predictive model could be developed to identify true responders from de novo resistance of androgen targeting therapeutics.

Patterns of PSA Changes After AR Signaling Directed Therapies

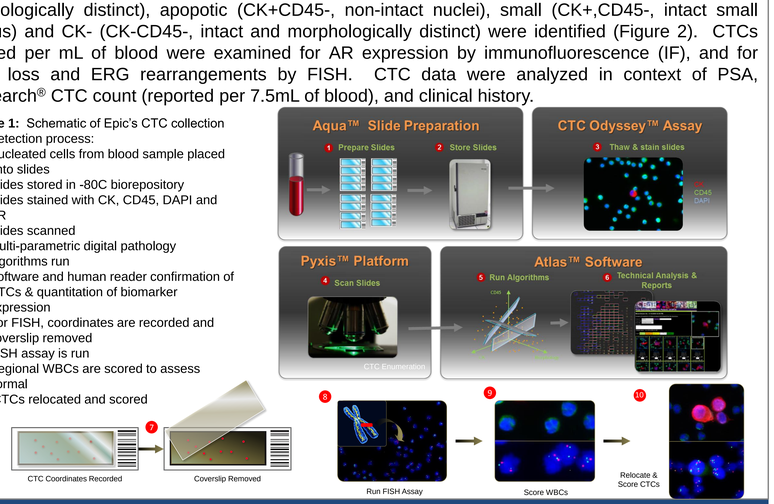


Methods

41 samples from 30 unique mCRPC pts treated with androgen receptor targeted (AR tx) therapies; 27 samples were from progressive pts and 14 samples from baseline pts. 24/27 (46.7%) of progressive patients were treated with Abiraterone plus Prednisone (AA+P) and 3/27 (53.3%) with Enzalutamide (E). Baseline samples were collected for 5/14 pts receiving AA+P, and 9/14 on E. Progressive pts were described as either de novo resistance, acquired resistance or true responders. Baseline pts were followed and received similar designation. Samples were collected and shipped to Epic Sciences where cells were stained and CTCs identified by fluorescent scanners and algorithmic analysis (Figure 1). CTCs, defined as traditional (CK+CD45- with intact DAPI nuclei and morphologically distinct), apopotic (CK+CD45-, non-intact nuclei), small (CK+,CD45-, intact small nucleus) and CK- (CK-CD45-, intact and morphologically distinct) were identified (Figure 2). CTCs reported per mL of blood were examined for AR expression by immunofluorescence (IF), and for PTEN loss and ERG rearrangements by FISH. CTC data were analyzed in context of PSA, CellSearch® CTC count (reported per 7.5mL of blood), and clinical history.

Figure 1: Schematic of Epic's CTC collection and detection process:

1) Nucleated cells from blood sample placed onto slides
2) Slides stored in -80C biorepository
3) Slides stained with CK, CD45, DAPI and AR
4) Slides scanned
5) Multi-parametric digital pathology algorithms run
6) Software and human reader confirmation of CTCs & quantitation of biomarker expression
7) For FISH, coordinates are recorded and coverslip removed
8) FISH assay is run
9) Regional WBCs are scored to assess normal
10) CTCs relocated and scored



Study Population

Table 1. Patient demographic and clinical characteristics at time of inclusion in the study.

Characteristic	No. (%) or Median (range)	Characteristic	No. (%) or Median (range)
Number of patients	30	**Metastatic Disease**	
Age, years	67.5 (47 – 85)	**Bone**	29 (97%)
Primary Treatment		**Lymph Node**	22 (73%)
Prostatectomy	15 (50%)	**Liver**	5 (17%)
Radiation	8 (27%)	**Lung**	3 (10%)
None	7 (23%)	**Other Soft Tissue**	3 (10%)
Therapies		**Laboratory Measures**	
Hormone Therapies		**PSA, ng/mL**	74.5 (1.9 - 9222)
1 -2 line	5 (17%)	**Hgb, (g/dl)**	11.9 (7.4 - 16)
3 lines	12 (40%)	**ALK, (unit/L)**	138 (54 - 562)
≥ 4 lines	13 (43%)	**LDH, (unit/L)**	243.5 (163 - 976)
Chemo-naïve	20 (67%)	**ALB, (g/dl)**	4.3 (3.6 - 5)
Chemo-exposed	10 (33%)	**CTC, (cells/7.5mL)**	6 (0 - >200)

CTC Frequency

Using the Epic Sciences CTC platform, CTC incidence was increased compared to CellSearch® CTC with 93% of samples accessed exhibiting >5 Epic "Traditional" CTCs/7.5mL. Additionally, novel CTC subpopulations were identified which demonstrated expression of PCa specific proteins and genomics (Figure 6) with 100% of patients exhibiting any CTC subpopulation at >5 CTC/7.5mL (Table 2 & Figure 2).

Table 2. Frequency of CTCs between sample types & platforms

Patients with >5 CTCs/7.5mL			
	CellSearch®	Epic "Traditional" CTC	Any Epic CTC Subtype
Progressive draw	15/27 (56%)	25/27 (93%)	27/27 (100%)
Baseline draw	8/14 (57%)	13/14 (93%)	14/14 (100%)
Total	23/41 (56%)	38/41 (93%)	41/41 (100%)

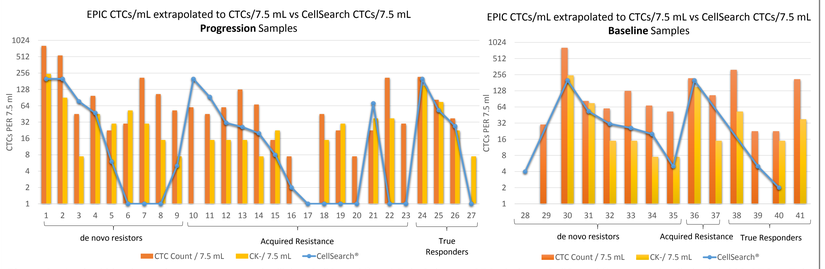


Figure 2. Matched blood samples were processed utilizing CellSearch® and Epic Sciences CTC platform. CellSearch® enumeration limited to 200 CTCs. Epic CTCs were measured from 1mL and extrapolated to 7.5 mL of blood.

CTC AR expression and CK- CTCs w/outcome

Mean AR expression of all CTC subtypes and frequency of CK- CTCs were measured in patients. Baseline draws from true responders had lower mean AR expression and fewer CK- CTCs (Table 3). Heterogeneity of CTC subtypes and AR expression were observed (Figure 4 & 5).

Table 3. Characteristics of AR expression and CK- CTCs by Progression and Baseline draw

		Cohort Characteristics	
Time point of Blood Draw	**Outcome**	**Cohort Average of Mean AR Expression of any CTC (Range)**	**Average CK- CTC frequency /7.5mL (Range)**
Progressive Draws (Patients w/rising PSA)	Initial True Responders n=4	4.13 (1.67-8.12)	63 (8-150)
	Initial Acquired Resistance n=14	3.13 (1.32-5.55)	14 (0-38)
	de Novo Resistance n=9	4.59 (1.46-12.25)	58 (8-248)
Baseline Draws (prior to AA+P or E)	True Responders n=4	1.76 (1.27-2.31)	17 (0-38)
	Acquired Resistance n=2	5.48 (2.83-8.12)	83 (15-150)
	de novo Resistance n=8	4.35 (1.63-12.25)	46 (0-248)

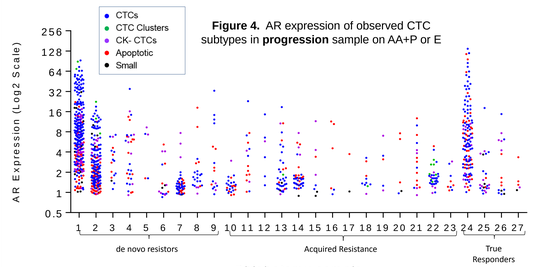


Figure 4. AR expression of observed CTC subtypes in **progression** sample on AA+P or E

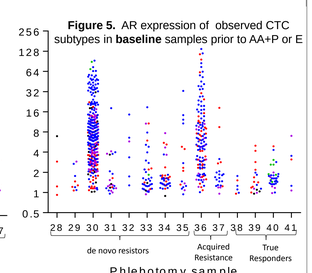
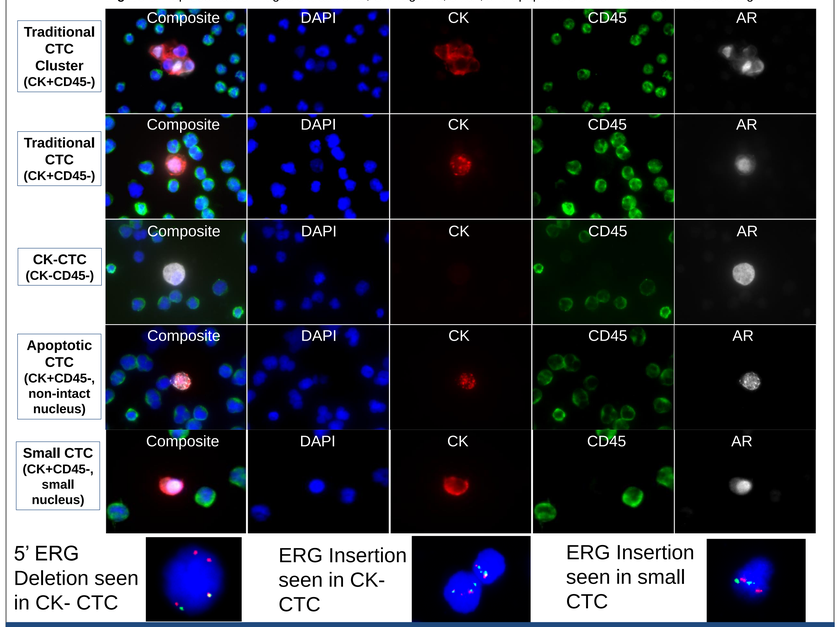


Figure 5. AR expression of observed CTC subtypes in **baseline** samples prior to AA+P or E

CTC Subpopulations detected on Epic Platform

Figure 6. Representative images of traditional, CK negative, small, and apoptotic CTCs. Inclusive of ERG rearrangement



5' ERG Deletion seen in CK- CTC

ERG Insertion seen in CK-CTC

ERG Insertion seen in small CTC

AR localization heterogeneity in de novo resistors

Example of AR Localization in CTCs

	Progressive Draws										Baseline Draws								
Patient ID	1	2	3	4	5	6	7	8	9		Patient ID	28	29	30	31	32	33	34	35
Nuclear	66%	36%	40%	31%	50%	0%	0%	0%	40%		Nuclear	N/A	0%	66%	30%	20%	4%	0%	40%
Equally Nuclear and Cytoplasmic	18%	4%	0%	0%	0%	0%	0%	0%	0%		Equally Nuclear and Cytoplasmic	N/A	0%	18%	0%	0%	4%	0%	0%
Cytoplasmic	6%	0%	10%	8%	0%	0%	0%	7%	0%		Cytoplasmic	N/A	0%	6%	0%	40%	12%	4%	0%
AR Negative	10%	61%	50%	62%	50%	100%	100%	93%	60%		AR Negative	N/A	100%	10%	70%	40%	81%	96%	60%

Conclusions

1. Epic CTC analysis provides higher detection rates than CellSearch® CTC in this cohort.
2. Novel CTC populations were detected in all (38) patients with 5 or more "traditional" CTC/7.5mL.
3. Notable was the marked heterogeneity of AR expression and subcellular localization in CTCs.
4. AR expression, heterogeneity and localization in CTCs along with frequency of CK- CTCs may play a role in progression of AR tx.
5. Studies are ongoing to quantitative measures we are reporting and undergoing further prospective validation.

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