Unique Patterns of the Selection and Change in Circulating Tumor Cell (CTC) Phenotypes and Genotypes by Drug Class in Metastatic Castration-Resistant Prostate Cancer (mCRPC)

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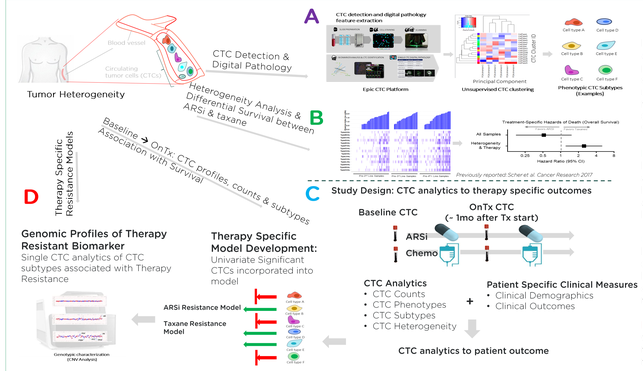
Background

Biomarkers to predict treatment outcomes for individual patients (pts) on standard of care Tx is an unmet medical need in the management of mCRPC. Using pre-treatment blood draws, we previously reported a phenotypic CTC heterogeneity algorithm that predicted differential survival times on ARSi vs. taxanes. We have also defined distinct phenotypic CTC subtypes to which different driver genomics and genomic instability are linked. We sought to correlate CTC phenotypes to drug sensitivity by determining OnTx CTC phenotypic and genotypic profiles to assess the effects of specific drug classes.

Methods: CTC Morphology and Heterogeneity Analysis



Description:

A) Epic CTC detection platform and unsupervised clustering of CTC subtypes using digital pathology features (i.e. Nuclear Area, Cytosolic Area, Nuclear Solidity)

B) Baseline CTC analysis of heterogeneity from Scher et al. 2017 previously identified a benefit to using taxanes over ARSi in patients with high CTC heterogeneity.

C) Study design. Paired baseline and OnTx blood draws were analyzed and CTC heterogeneity, subtypes, and overall counts were compared to clinical outcomes.

D) CTC subtypes associated with clinical outcome are used to create Tx specific models of treatment resistance.

Patient Demographics and Study Design

Patient Characteristic	ARSi (N = 117)	Taxane (N= 94)	Platinum (N=34)
Tx Line - no. (%)			
Pre 1st	42 (35.90%)	8 (8.51%)	8 (22.86%)
Pre 2nd	48 (41.03%)	15 (15.96%)	4 (11.43%)
Pre 3rd+	27 (23.08%)	71 (75.53%)	22 (62.86%)
Median Age in yr (min, max)	69 (45,87)	68 (48,91)	67 (44,82)
Median ALB in g/dL (min, max)	4.2 (3.3,4.9)	4.15 (3.1,4.9)	4.1 (3.4,4.6)
Median ALK in U/L (min, max)	92.5 (42,2170)	135. (43,1055)	120 (51,1043)
Median HGB in g/dL (min, max)	12.3 (7,15.1)	11.2 (8,14.7)	11.3 (6.3,14.1)
Median LDH in U/L (min, max)	202 (124,2115)	254 (141,1487)	283. (155,799)
Median Baseline PSA in ng/mL (min, max)	20.4 (0.09,2006)	99.4 (0.09,1627)	30.5 (0,1120)
Metastases - no. (%)			
Bone	93 (79.49%)	89 (94.68%)	NA
Liver	5 (4.27%)	15 (15.96%)	NA
Lung	7 (5.98%)	12 (12.77%)	NA

Patient summary
246 Baseline and OnTx blood draws from three therapy classes

1) 117 ARSi treated pts
2) 95 Taxane treated pts
3) 34 Platinum treated pts

Metrics
Changes in CTC number, morphology, and heterogeneity in post- relative to pre-therapy samples using the Epic Sciences platform

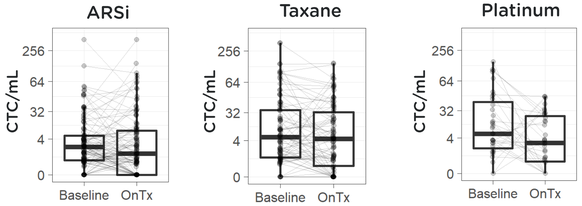
Endpoint
Comparison to overall survival

CTC Counts OnTx are Independent of Baseline Counts

A

	ARSi (N= 117)	Taxane (N=94)	Platinum (N=34)
Baseline CTC/mL > 0 (no.)	100 (85.47%)	85 (90.43%)	32 (91.43%)
Median Baseline CTC/mL (min, max)	2.5 (0,420)	4.77 (0,365.)	5 (0,150)
OnTx CTC/mL > 0 (no.)	85 (72.65%)	75 (79.79%)	27 (77.14%)
Median OnTx CTC/mL (min, max)	1.56 (0,415)	4.34 (0,833.)	2.98 (0,31)
Median OnTx Draw Time from Tx start in mo (min, max)	0.98 (0.23,4.6)	0.69 (7,140)	0.88 (0.23,3.7)
# of Pts with a CTC increase	48	39	9
# of PTs with a CTC decrease	62	49	25
# of Pts with no CTCs at Baseline and OnTx	7	6	0

B

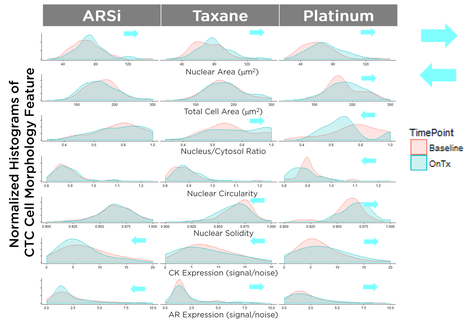


Description:

A) Table of median CTC counts and number of Pts with a CTC increase, decrease or that have zero CTCs at both Baseline and OnTx.

B) Boxplot of CTC/mL counts at baseline and OnTx by Tx class.

Therapy Specific CTC Cell Morphology Changes are Observed Between Patients Treated with ARSi, Taxanes and Platinum



Digital pathology feature trends towards an increase OnTx

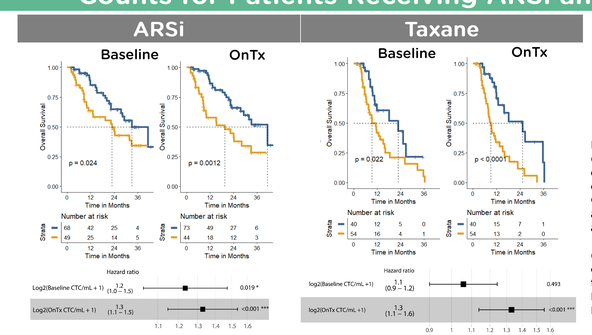
Digital pathology feature trends towards a reduction OnTx

#CTCs analyzed

	Baseline	OnTx
ARSi	2595	2186
Taxane	3045	2301
Platinum	927	351

Description:
Cell morphology features of each CTC identified were extracted and plotted as a normalized histogram comparing the overall change from baseline to OnTx for ARSi, Taxanes and Platinum.

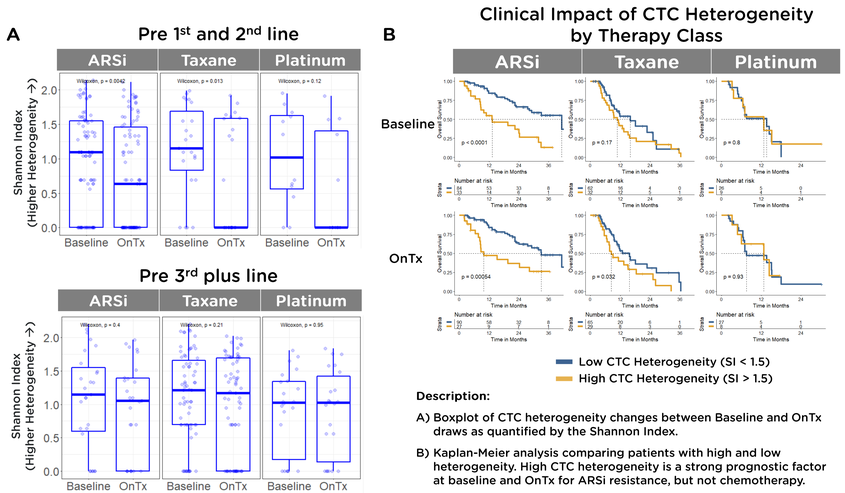
OnTx CTC Counts Associate with Worse Survival Than Baseline Counts for Patients Receiving ARSi and Taxanes



Description:
(Top) Kaplan-Meier analysis demonstrating that OnTx CTC counts are more prognostic of poor OS than baseline counts. This was also confirmed in a multivariate analysis.

(Bottom) Multivariate comparison of OnTx and Baseline CTCs as log2 transformed continuous variables. Hazard ratios are for each OnTx or Baseline CTC doubling.

CTC Heterogeneity Decreases Following Treatment in Patients Receiving 1st and 2nd Line, But Not 3rd Line Therapy



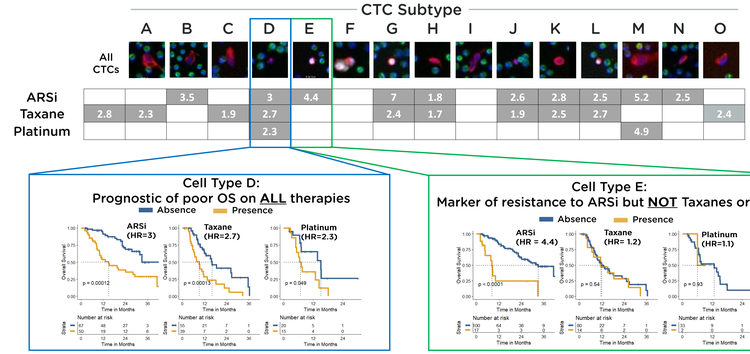
Description:

A) Boxplot of CTC heterogeneity changes between Baseline and OnTx draws as quantified by the Shannon Index.

B) Kaplan-Meier analysis comparing patients with high and low heterogeneity. High CTC heterogeneity is a strong prognostic factor at baseline and OnTx for ARSi resistance, but not chemotherapy.

Presence of Specific OnTx CTC Cell Types Associated with Therapy Resistance and Poor OS

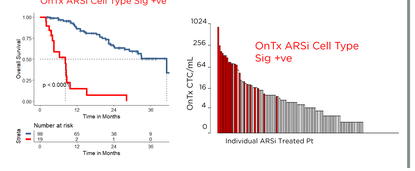
Table of univariate Hazard Ratios comparing absence versus presence of each cell type within the OnTx blood draw (only Hazard Ratios with log-rank significance < 0.05 are shown)



Presence of Specific OnTx CTC Cell Types Are Prognostic of Therapy Resistance and Poor OS

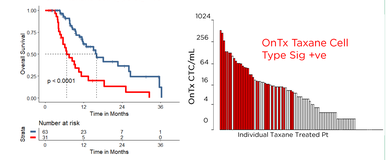
ARSi Resistance Model

ARSi Cell Type Signature (Presence of more than 3 of the cell types D, E, G, K, L, M, N)



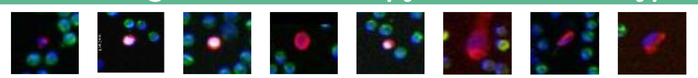
Taxane Resistance Model

Taxane Cell Type Signature (Presence of more than 3 of the cell types D, G, K, L, O)



Description:
A) Kaplan-Meier curves comparing patients positive and negative for the ARSi (Left) or Taxane (Right) cell type signature. Univariate hazard ratios were validated in a multivariate model including clinical variables.
B) Relationship between total CTC/mL and cell type signature positivity.

Single Cell Sequencing of 1569 CTCs Identifies Unique Genomic Signatures of Therapy Resistant Cell Types



	D	E	G	K	L	M	N	O
Gain	ATM	AR	MYC	YWHAZ	RFC2	AURKA	BRCA1	FCMR
	KLK2	AKT3	UTP20	AURKA	MYC	BMI1	CDKN1B	ATM
	CHTOP	BRAF	PTK2	SPOP	AR	BRAF	IFT81	AR
Loss	SPOP	CCDC69	SFTPC	NABP2	SFTPC	RB1	BRCA2	PTEN
	PTK2B	BRCA1	TNFRSF10A	LOC284294	CDH1	NABP2	LOC284294	TNFRSF10A
	KLF3	SFTPC	LINC01572	RB1	WWOX	BCL2	APC	NKX3-1

Description
1569 CTC subtypes were single cell sequenced and the percentage of CTCs with specific genomic amplifications and deletions were determined. The top three most significant genes copy number gains and losses from each CTC subtype are shown.
Observations
Cell Type E associated with ARSi resistance has the highest percentage of AR amplification

Conclusions

- Post treatment changes in specific CTC subtypes vary by drug class.
- CTC count and CTC subtypes are present in OnTx blood samples. Presence of OnTx CTCs are generally associated with worse overall survival compared with CTCs in Baseline draws
- CTC heterogeneity and discrete CTC subtypes impact patient survival by Tx class
- Models to improve prediction of therapeutic benefit can be developed utilizing CTC phenotypic profiling