Phenotypic Circulating Tumor Cell (CTC) Classifier of Genomic Instability (GI) Associates with Improved Overall Survival (OS) for Metastatic Castration-Resistant Prostate Cancer (mCRPC) Patients (pts) Receiving Platinum Agents in Addition to Taxanes

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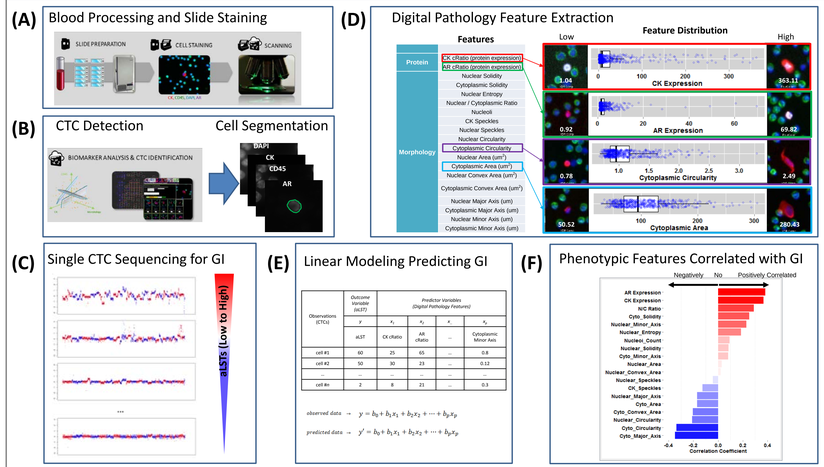
Background

- The presence of Genomic Instability (GI) has been associated with DNA Damage Response (DDR) genetic profile. mCRPC pts with DDR are responsive to poly ADP ribose polymerase inhibitors (PARPi).
- Similar Tx benefit for tumors with DDR(+) profiles has been observed with alkylating agents such as platinum Tx in small cohorts in triple-negative breast cancer and prostate cancer (Pomerantz, JCO, 2017).
- Predictive biomarkers to identify pts with improved outcomes on platinum Tx are needed, but obtaining and sequencing metastatic biopsies to identify DDR(+) tumors is not scalable for routine clinical use due to accessibility, heterogeneity, cost and time to result.
- Previously we developed an imaging-based phenotypic classifier to predict GI (pGI) from individual CTC morphology and demonstrated that these pts with pGI(+) had inferior survival times when treated with androgen receptor signaling inhibitors (ARSi) or Taxanes (Scher et al. ASCO 2016). The same classifier also predicted improved PSA response rate when pts with pGI(+) CTCs received PARPi + ARSi vs. ARSi alone (Feng et al ESMO 2016).
- Here we analyzed if: **1) the same classifier could predict improved OS in pts receiving taxane + platinum vs. taxane alone. 2) pGI(+) CTCs were more likely to be eliminated on Tx due to platinum chemotherapy activity**

Methods for CTC Detection and Characterization

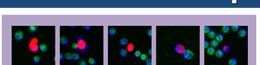


Observations (CTCs)	Outcome Variable (aSTs)	Predictor Variables (Digital Pathology Features)			
	y	x_1	x_2	x	x_p
	aSTs	CK cRatio	AR cRatio	...	Cytoplasmic Minor Axis
cell #1	60	25	15	...	0.8
cell #2	50	30	23	...	0.12
...	...	...	...	...	...
cell #n	2	8	21	...	0.3

Schematic of Epic CTC genomic & phenotypic characterization, biomarker prediction workflow:

A. Nucleated cells from blood sample placed onto slides and then stored in a -80˚C biorepository. Slides stained with cytokeratin (CK), CD45, DAPI, and Androgen Receptor (AR). Slides scanned.
B. CTC candidates detected by a multi-parametric digital pathology algorithm, technician confirmed, and CTCs segmented.
C. CTCs picked and sequenced for copy number variations (CNV). Number of break points with chromosomal segments larger than 10MB were counted as large scale transitions (LSTs). Wide range of LSTs (0 - 60+) observed in mCRPC pts.
D. Segmentation algorithms isolate pixels corresponding to each CTC in all 4 individual channels and digital pathology features per CTC are extracted from segmented pixels.
E. Linear regression model was built and trained to predict LSTs (from sequencing) using digital pathology features (from imaging).
F. Bar graph showing Pearson correlation coefficients of morphological features to LSTs. Y-axis shows morphological features and X-axis shows correlation coefficients (*r*). From top to bottom, features were ranked by *r* values (high to low) and bar length represents absolute *r* values.

Example CTCs: pGI(+) and pGI(-)



Predicted Positive for Genomic Instability

- High nuclear/cytoplasm ratio
- Low eccentricity
- High nuclear texture

Predicted Negative for Genomic Instability

- Normal nuclear/cytoplasm ratio
- High eccentricity
- Normal nuclear texture

IF Staining:

- Red = Cytokeratins
- blue = DAPI (DNA)
- green = CD45

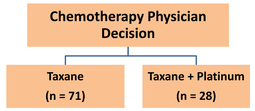
Patient Selection and Demographics

CTC Monitoring Cohort (Paired samples: pre-Tx and on-Tx)

Tx		Paired Samples
Taxane Only (n=57)	Taxane Only	57
Platinum (Single or Combo) (n=39)	Taxane + Platinum	25
	Etoposide + Platinum	8
	Platinum alone	6

	Taxane Only	Taxane & Platinum	Etoposide & Platinum	Platinum Only
Paired Samples	57	25	8	6
Median Survival (months)	16.3	13	6.5	7.2
Median PSA	99.5	30.5	3.9	202.9
Median LDH	250	293	290	283
Median Albumin	4.2	4.2	4.1	3.7
Medium Hemoglobin	11.4	11.5	10.4	10.3
Median Alk Phos	156	165	77.5	134
Median WBC	6.1	7.2	6.1	6.6
Median CK(+) CTC/mL	3.9	3.5	2.1	11.8
Median AR(+) CTC/mL	1.2	0	0	0.4

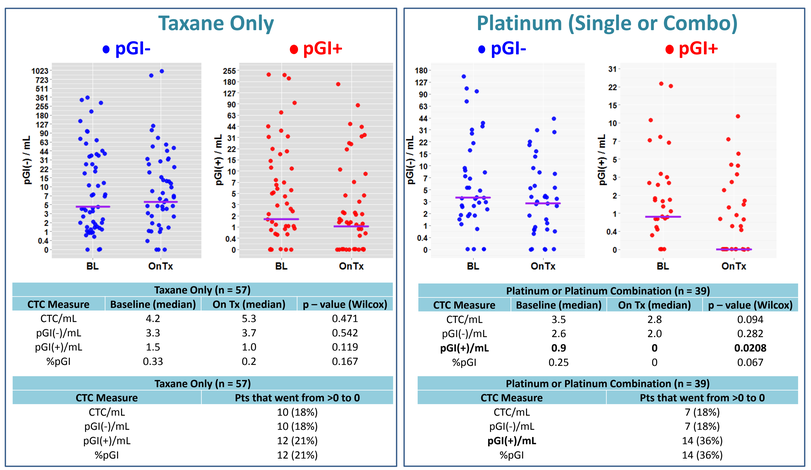
Outcome Association Cohort (pre-Tx samples only)



	Taxane Only	Taxane & Platinum
Samples	71	28
Median Survival (months)	13.0	8.8
Median PSA	86.1	33.1
Median LDH	257.0	283.5
Median Albumin	4.2	4.2
Medium Hemoglobin	11.4	11.5
Median Alk Phos	173.0	154.0
Median WBC	6.7	7.1
Median CK(+) CTC/mL	3.8	4.4
Median AR(+) CTC/mL	1.2	0.0

- CTC Monitoring Cohort utilized all available samples with both a baseline (pre-Tx) and Tx draw for pts receiving taxanes or platinum agents
- Outcome Association Cohort utilized all samples taken prior to either taxane administration, or combination of taxanes and platinum agents. Choice of Tx was at the discretion of the attending physician without CTC results. Pts receiving combination of platinum and taxanes generally had lower PSA

pGI(+) CTCs decrease on Platinum Tx but not Taxane Tx Alone



Taxane Only (n = 57)			
CTC Measure	Baseline (median)	On Tx (median)	p – value (Wilcox)
CTC/mL	4.2	5.3	0.471
pGI(-)/mL	3.3	3.7	0.542
pGI(+)/mL	1.5	1.0	0.119
%pGI	0.33	0.2	0.167

Taxane Only (n = 57)	
CTC Measure	Pts that went from >0 to 0
CTC/mL	10 (18%)
pGI(-)/mL	10 (18%)
pGI(+)/mL	12 (21%)
%pGI	12 (21%)

Platinum or Platinum Combination (n = 39)			
CTC Measure	Baseline (median)	On Tx (median)	p – value (Wilcox)
CTC/mL	3.5	2.8	0.094
pGI(-)/mL	2.6	2.0	0.282
pGI(+)/mL	**0.9**	**0**	**0.0208**
%pGI	0.25	0	0.067

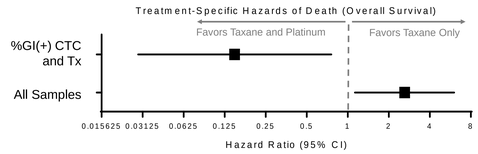
Platinum or Platinum Combination (n = 39)	
CTC Measure	Pts that went from >0 to 0
CTC/mL	7 (18%)
pGI(-)/mL	7 (18%)
pGI(+)/mL	14 (36%)
%pGI	14 (36%)

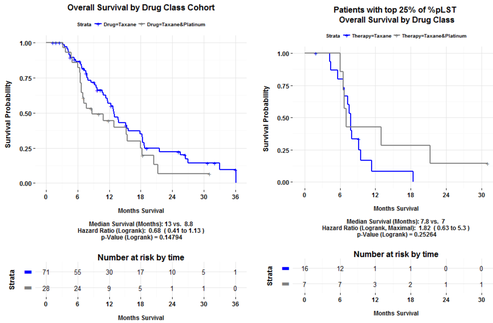
- It has been reported that cells with genomic instability (manifested through DNA repair deficiencies) are preferentially sensitive to DNA damaging agents like platinum agents
- We hypothesized that the genomic instability phenotype would be preferentially reduced from baseline to samples taken on platinum Tx (or platinum Tx combinations) compared to taxanes only

High %pGI(+) CTCs Might be Associated with Improved OS when Platinum added to Taxanes

Multivariable Cox Proportional Hazard Analysis of Predictors of Overall Survival		
Effect	**P-value**	**HR (95% CI)**
CK(+) CTC Count (log, continuous)	0.0302	1.17 (1.01 to 1.34)
%pGI (continuous)	0.0379	2.96 (1.06 to 8.25)
Taxane and Platinum vs. Taxane Only	0.0236	2.62 (1.13 to 6.04)
Tx Interaction: Increasing %pGI on T&P vs. Taxane Only	0.0220	0.148 (0.0286 to 0.759)

- These are retrospective analyses of prospectively banked samples
- Pt groups were not randomized; pts receiving platinum agents generally had more aggressive disease
- The relationship between CTC burden, %pGI(+) CTCs, and Tx class with OS was explored with a Cox PH model
- The results suggest that in a properly balanced cohort, pts with high %pGI(+) might have better OS if platinum agents are added to taxanes
- Corroborating this result is the observation that some pts with high %pGI(+) CTCs remained alive for an extended period of time compared to the rest of the cohort (far right)





Conclusions

- Digital Pathology can predict presence of genomic instability of individual CTCs without sequencing pt tumor samples.
- %pGI(+) CTCs have a Tx interaction (HR=0.148, *p*=0.022) with improved outcome with taxane + platinum over taxane alone, suggesting that a properly balanced cohort might show improved OS for platinum use in pts with high %pGI(+) CTCs.
- Pts receiving platinum chemotherapy alone or in combination, but not taxanes alone, are likely to have a drop of pGI(+) CTCs after Tx initiation, suggesting that the pGI(+) phenotype is preferentially sensitive to platinum containing therapeutic regiments.
- Clinical validation of the utility of pGI(+) CTCs to predict sensitivity to platinum and PARPi agents, and the association with improved pt outcomes, are underway.

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