Phenotypic and genomic characterization of CTCs as a biomarker for prediction of Veliparib therapy benefit in mCRPC

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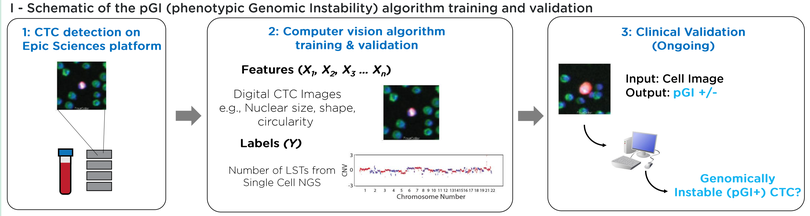
Background

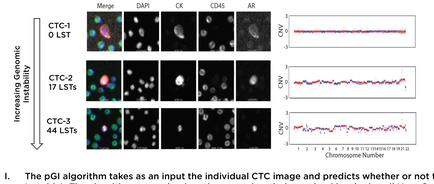
The NCI 9012 study, which randomized 1st line mCRPC patients to Abiraterone or Abiraterone + Veliparib, demonstrated no advantage for Veliparib in any pre-specified subgroup, with DNA damage response mutations (DDRm) pts having better outcomes on both arms. This contrasted with the TOPARP trial[1], which enrolled heavily pre-treated pts with high CTC counts, where responses to PARPi were observed, particularly in patients with DDRm. Here we explore the use of CTCs in the NCI 9012 study to ascertain phenotypic Genomic Instability (pGI) as a proposed selection biomarker for PARPi.

The pGI CTC Biomarker Methodology

I - Schematic of the pGI (phenotypic Genomic Instability) algorithm training and validation

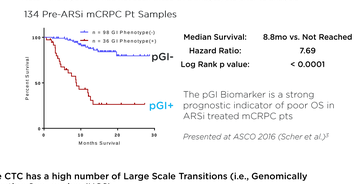


II - Example CTC images and corresponding single cell sequencing from the training set



III – Prognostic value of pGI status at Baseline (mCRPC ARSi treated pts)

*Note – the algorithm had not seen these CTC images/pts prior to analysis



Median Survival:	8.8mo vs. Not Reached
Hazard Ratio:	7.69
Log Rank p value:	< 0.0001

The pGI Biomarker is a strong prognostic indicator of poor OS in ARSi treated mCRPC pts

Presented at ASCO 2016 (Scher et al.)[2]

I. The pGI algorithm takes as an input the individual CTC image and predicts whether or not the CTC has a high number of Large Scale Transitions (i.e., Genomically Instable). The algorithm was trained on the ground truth determined by single cell Next Generation Sequencing (NGS).

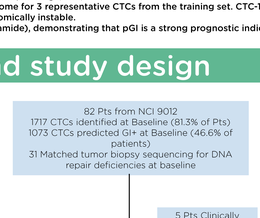
II. Example CTC images and corresponding single cell copy number variation (CNV) plots by chromosome for 3 representative CTCs from the training set. CTC-1 has a 'flat' or unperturbed genome while CTC-2 and CTC-3 have a high number of LSTs and are therefore, genomically instable.

III. Independent testing of pGI in a cohort of mCRPC Pts treated with ARSi Rx (Abiraterone or Enzalutamide), demonstrating that pGI is a strong prognostic indicator of poor OS.

Patient demographics and study design

Patient Characteristic	Arm A (Abi) N=35	Arm B (Abi + Veliparib) N= 40	Significance	p-value*
Median Age in yr (min, max)	67.2 (50.6,79.8)	68.1 (53.2,85.8)	NS	0.85
CTC/mL > 0	29 (82.86%)	32 (80.00%)	NS	1.00
Median CTC/mL (min, max)	2.8 (0,259)	3.8 (0,287)	NS	0.55
pGI+ CTC/mL > 0	12 (34.29%)	23 (57.50%)	NS	0.07
Median pGI+ CTC/mL (min, max)	0 (0,129)	1.0 (0,199)	NS	0.37
Race - no. (%)			NS	0.50
White	32 (91.43%)	37 (92.50%)		
Black	2 (5.71%)	1 (2.50%)		
Other	1 (2.86%)	2 (5.00%)		
ECOG - no. (%)			NS	0.32
0	22 (62.86%)	29 (72.50%)		
1	13 (37.14%)	10 (25.00%)		
2	0 (0.00%)	1 (2.50%)		
Median PSA ng/mL (min, max)	28.84 (0.8,459.0)	37.32 (0.04,802.0)	NS	0.24
Cancer Pain Present - no. (%)	12 (34.29%)	10 (25.00%)	NS	0.58
Sites of Disease - no. (%)				
Lymph Nodes	21 (60.00%)	28 (70.00%)	NS	0.25
Bone	32 (91.43%)	35 (87.50%)	NS	0.71
Visceral	8 (22.86%)	11 (27.50%)	NS	0.60
Other	6 (17.14%)	9 (22.50%)	NS	0.57
Previous Treatments - no. (%)				
Chemotherapy	5 (14.29%)	12 (30.00%)	NS	0.21
Enzalutamide	1 (2.86%)	0 (0.00%)	NS	0.48
Experimental Agent	9 (25.71%)	4 (10.00%)	NS	0.08
Sipuleucel-T	10 (28.57%)	7 (17.50%)	NS	0.41
ETS Fusion - no. (%)			NS	1.00
Positive	14 (40.00%)	16 (40.00%)		
Negative	21 (60.00%)	24 (60.00%)		
Previous Ketoconazole - no. (%)	3 (8.57%)	4 (10.00%)	NS	1.00
Median Treatment Cycles (min, max)	9 (2,39)	12 (1,38)	NS	0.46
Confirmed PSA Response - no. (%)	22 (62.86%)	30 (75.00%)	NS	0.32
Confirmed PSA Progression - no. (%)	25 (71.43%)	26 (65.00%)	NS	0.64
Median Time to PSA Progression (Months)	8.57 (0.89,22.9)	11.03 (0.919,25.3)	NS	0.27
Measurable disease at baseline - no. (%)	20 (57.14%)	25 (62.50%)	NS	0.49
Median PFS (Months)	8.28 (0.89,22.9)	11.02 (0.919,25.3)	NS	0.44

*p-values calculated using fisher's exact test for categorical variables and Welch's t-test for continuous variables



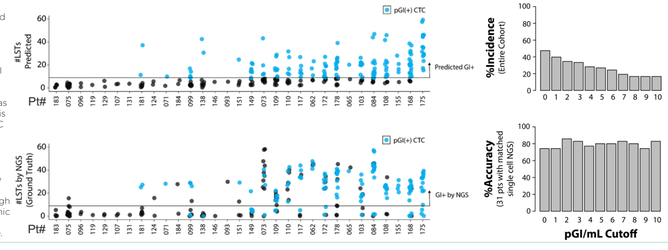
Analytical performance of the pGI biomarker

Experiment

- A subset of 35 Baseline blood draws from unique Pts had their CTCs both single cell sequenced by NGS and their CTC images run through the pGI prediction algorithm. pGI positive cells are colored in cyan. LST = Large Scale Transitions. The algorithm was locked prior to analysis of this cohort and had not seen CTC images from this cohort during training.

Conclusion

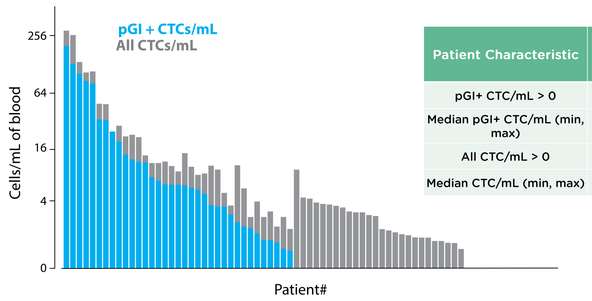
- The pGI algorithm accurately identified pts whose blood contains CTCs that have a high number of large scale genomic transitions – i.e., those CTCs that are genomically instable.



References

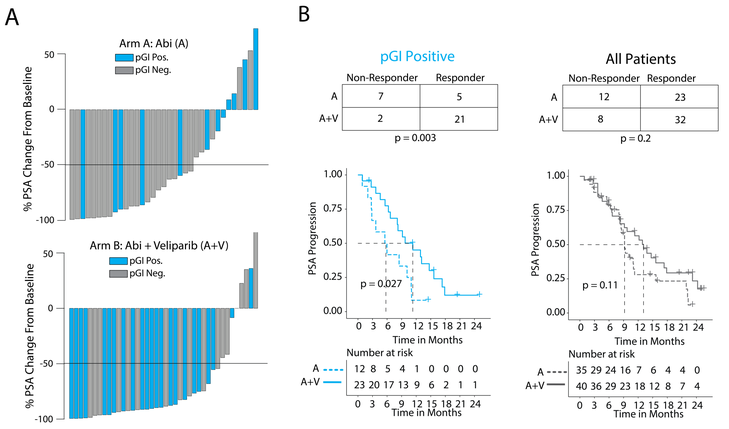
1. Mateo, Joaquin, et al. "DNA-repair defects and olaparib in metastatic prostate cancer." New England Journal of Medicine373.18 (2015): 1697-1708.
2. Scher, Howard I., et al. "CTC phenotype classifier to identify mCRPC patients (pts) with high genomic instability CTCs and to predict failure of androgen receptor signaling (AR Tx) and taxane (T) systemic therapies." (2016): 5044-5044.

pGI+ and total CTC prevalence



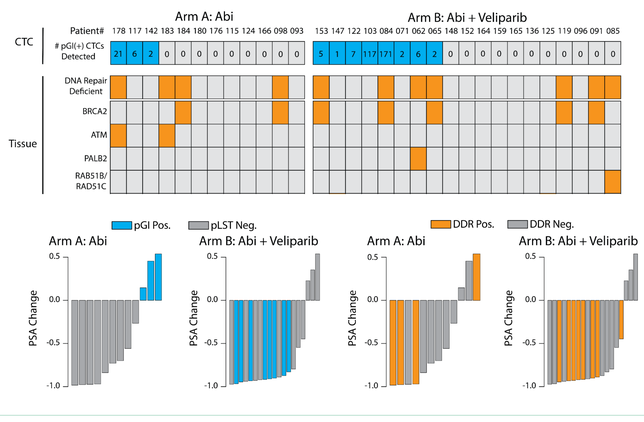
Patient Characteristic	Arm A (Abi) N=35	Arm B (Abi + Veliparib) N= 40
pGI+ CTC/mL > 0	12 (34.29%)	23 (57.50%)
Median pGI+ CTC/mL (min, max)	0 (0,129)	1.0 (0,199)
All CTC/mL > 0	29 (82.86%)	32 (80.00%)
Median CTC/mL (min, max)	2.8 (0,259)	3.8 (0,287)

pGI positive patients associate with improved response in the Abi + Veliparib Tx arm vs. Abi alone



A) PSA waterfall. B) Kaplan-Meier analysis and Fisher's exact test comparing PSA responders and non-responders by Tx arm. Any patient having greater than 0 pGI+ CTCs is defined as pGI positive.

CTC pGI and Tissue DDRm relationship to outcome

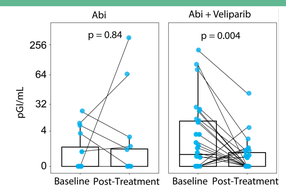


pGI+ CTCs decrease after start of Abi + Veliparib but not Abi alone

Analysis of longitudinal changes of pGI+ CTCs were examined in 46 matched baseline and on-therapy samples (n=92 total blood samples).

	Abi	Abi + Veliparib
pGI+ CTC Increase or No Change	13	13
Decrease	3	17

p = 0.03

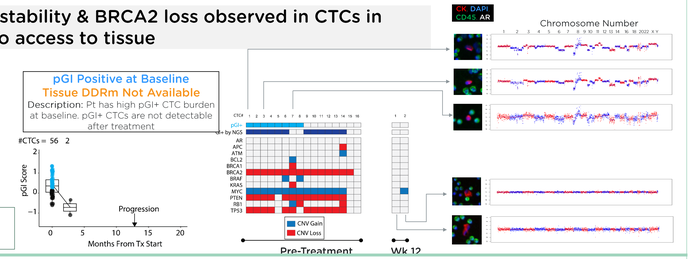


Patient Examples of Single CTC Genomic Instability and Clonal Heterogeneity & Relationship to Outcome

Genomic Instability & BRCA2 loss observed in CTCs in patients w/o access to tissue

pGI Positive at Baseline
Tissue DDRm Not Available
Description: Pt has high pGI+ CTC burden at baseline. pGI+ CTCs are not detectable after treatment

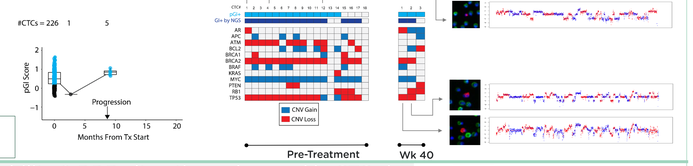
Responder to: Abi + Veliparib



DDR Genomic alterations observed in CTC before and at progression of Abi + Veliparib Tx

pGI Positive
Tissue DDRm Positive
Description: Pt has high pGI+ CTC burden at baseline. pGI+ CTCs return, Pt progresses

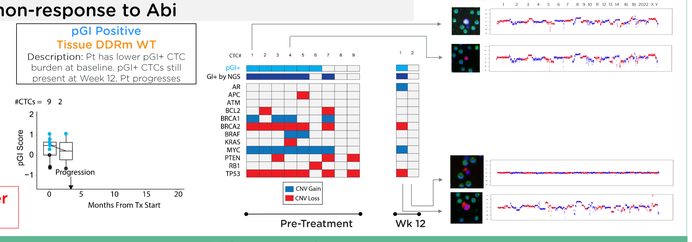
Responder to: Abi + Veliparib



Genomic Instability & DDR alterations observed in CTCs in patient with non-response to Abi

pGI Positive
Tissue DDRm WT
Description: Pt has lower pGI+ CTC burden at baseline. pGI+ CTCs still present at Week 12. Pt progresses

Non-Responder to: Abi



Discussion

- Phenotypic Genomic Instability (pGI) CTC algorithm identifies single cell and patients with true genomic instability
- Patients with pGI+ CTCs receiving Abiraterone + Veliparib vs. Abiraterone alone are associated with improved PSA response
- pGI CTCs have a significant reduction from baseline to Post-Tx-start in Abiraterone + Veliparib but not in Abiraterone only
- Single CTC genomic analysis supports biologic mechanism of response & resistance to Veliparib & Abiraterone