Prediction of PARP inhibitor response and resistance utilizing a CTC phenotypic classifier in patients with metastatic castration-resistant prostate cancer (mCRPC):Results from the NCI 9012 trial

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Background

PARP inhibitors (PARPi) have efficacy in mCRPC harboring homologous recombination DNA repair deficiencies (HRD), but there is no non-invasive assay to predict PARPi response.[1] Biomarkers of HRD, including genomic scarring or alterations of HRD related genes, have demonstrated an ability to predict PARPi patient response.[2] However, despite HRD related genomics, not all patients respond, while many patients without HRD genomics do. In addition, the identification of secondary mutations identify cells ability to become HRD competent, PARP reversion.[3] Previous work characterizing single CTCs from mCRPC patients has identified subclonal populations of CTCs with unique phenotypes and somatic genomic instability consistent with HRD and resulting in worse outcomes following Abiraterone treatment.[4] NCI 9012 evaluated Abiraterone alone with or without the PARPi Veliparib in mCRPC. We now determine if we can identify PARPi resistant CTC phenotypic subtypes and when combined with previously trained HRD CTC phenotypes can develop a biomarker which identifies patients who will have improved outcomes with Veliparib + Abiraterone vs. Abiraterone alone.

Methods for CTC Detection; Phenotypic, Genomic Characterization
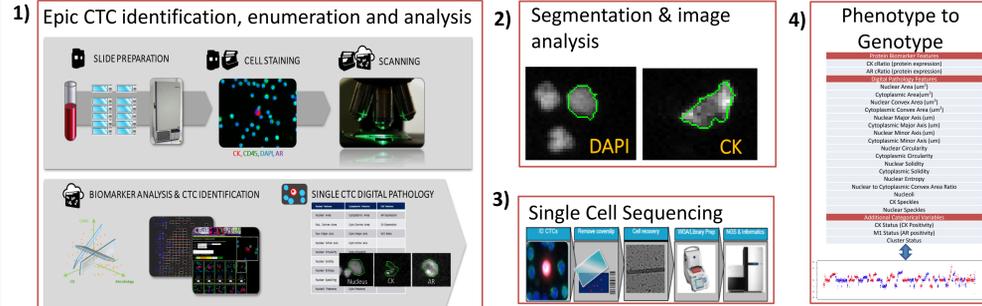
1) Epic CTC identification, enumeration and analysis: Nucleated cells from blood sample placed onto slides and stored in a -80°C biorepository. Slides are stained with cytokeratin (CK), CD45, DAPI, AR N-term and scanned. CTC candidates are detected by a multi-parametric digital pathology algorithm followed by human reader confirmation of CTCs and quantification of biomarker expression.
2) Segmentation and image analysis: CTCs are segmented within the DAPI, CK, AR channels and ~20 nuclear and morphological features are extracted.
3) Single Cell Sequencing: Individual CTCs representing unique morphological phenotypes are recovered from the surface of the slide post morphological analysis. As per previously presented[5], single CTCs are lysed, whole genome amplified and constructed into shotgun libraries. Each library is sequenced by low pass whole genome (0.3X). Whole genome CNV categorizes 1Mb segments for amplifications or deletions. Regions >10Mb combined gained or loss are scored as a single large scale transition (LST)[6].
4) Phenotype to genotype analysis: As previously described[4],quantitative and qualitative digital pathology features were correlated with the magnitude of quantified, sequenced-derived actual LST (aLST) per CTC using regression modeling. The output of this model is dichotomized and identified as an HRD+ phenotype CTC.

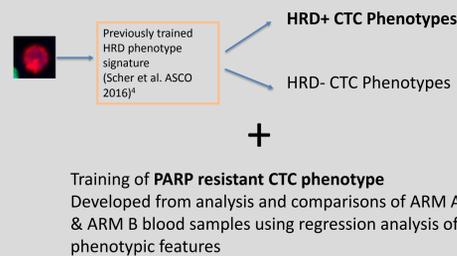## Patient Demographics & CTC Classifier Development

NCI 9012 Clinical Trial Demographics

NCI 9012 Demographics	Number (%)
Patients registered (12 sites)	190
Race of Patient	
White	164 (86%)
Black/Other	26 (14%)
PSA/Age Demographics	
Median Age (Min, Max)	68 (45-90)
Median PSA (Min, Max)	35.5 (0.04-1558)
Biopsy Type	
Patients who underwent metastatic biopsy	185
Bone	96 (52%)
Soft Tissue	89 (48%)

Blood Samples for CTC analysis

* 2/84 baseline sample not utilized due to insufficient follow-up clinical data

Components of the CTC Classifier

Previously trained HRD phenotype signature (Scher et al. ASCO 2016)[4]

→ HRD+ CTC Phenotypes
→ HRD- CTC Phenotypes

+

Training of PARP resistant CTC phenotype
Developed from analysis and comparisons of ARM A & ARM B blood samples using regression analysis of phenotypic features

PARPi CTC Biomarker Classifier Definition

Biomarker classification	Biomarker Definition
Biomarker +	Presence of HRD+ CTCs & Absence of PARP resistant CTCs
Biomarker -	Presence of PARP resistant CTCs or absence of HRD+ CTCs

References: (1) Mateo, Joaquin, et al. New England Journal of Medicine 373.18 (2015): 1697-1708. (2) Fong, Peter C., et al. New England Journal of Medicine 361.2 (2009): 123-134. (3) Cruz, C., et al. Cancer Research 76.4 Supplement (2016): P4-07. (4) Scher, Howard I., et al. ASCO Annual Meeting Proceedings. Vol. 34. No. 15_suppl. 2016. (5) Landers, Mark, et al. ASCO Annual Meeting Proceedings. Vol. 33. No. 15_suppl. 2015. (6) Popova, Tatiana, et al. Cancer research 72.21 (2012): 5454-5462.

Phenotypic & Genomic Profiles of CTCs

HRD- CTCs Phenotypes
- Low nuclear/cytoplasm ratio
- High eccentricity
- Low nuclear texture

HRD+ CTCs Phenotypes
- High nuclear/cytoplasm ratio
- Low eccentricity
- High nuclear texture

PARP resistant CTC Phenotypes
- Normal nuclear/cytoplasm ratio
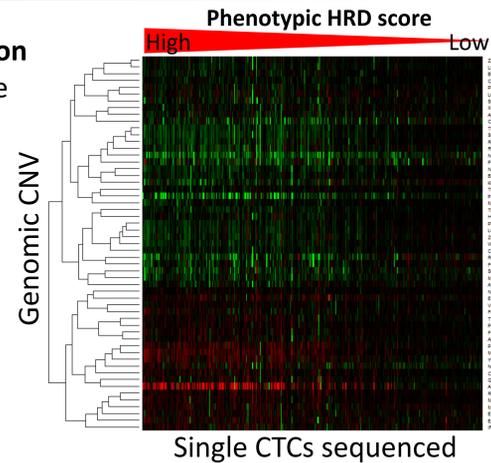- High eccentricity
- High nuclear area
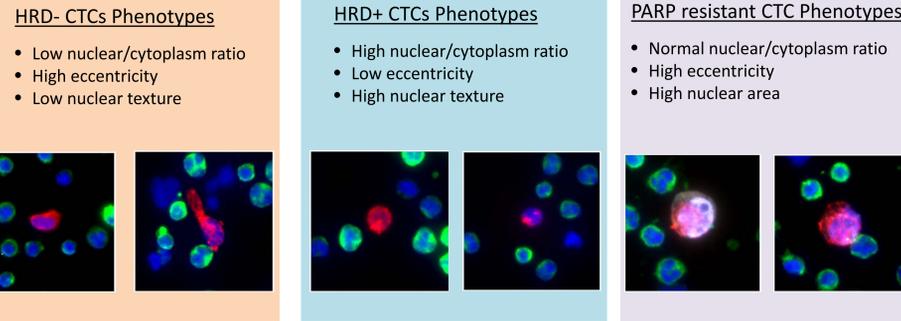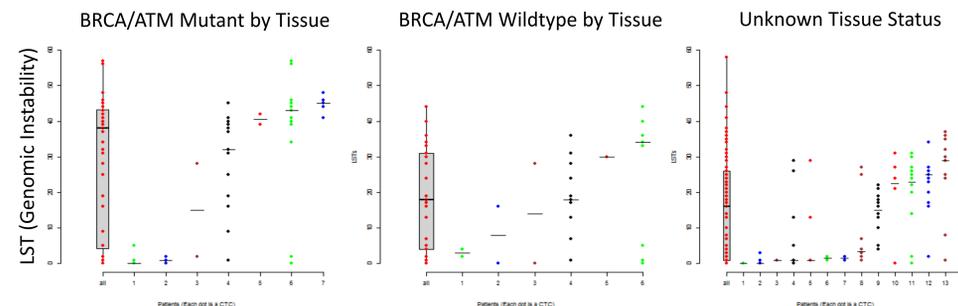

Phenotype → Genotype Association
HRD+ Phenotypic CTC scores have increased Genomic Instability

434 CTCs from 35 patients were measured for HRD Phenotype utilizing a previous trained algorithm in an independent cohort (Scher et al.)[4]

Visualized are the 434 single CTC CNV genomic alterations (green=deletion; red=amplification) of known oncogenes and tumor suppressors, ranked from highest (left) to lowest (right).

Phenotypic HRD score — High ... Low
Genomic CNV — Single CTCs sequenced

Association of Single CTC Genomics to Tissue Sequencing

BRCA/ATM Mutant by Tissue | BRCA/ATM Wildtype by Tissue | Unknown Tissue Status
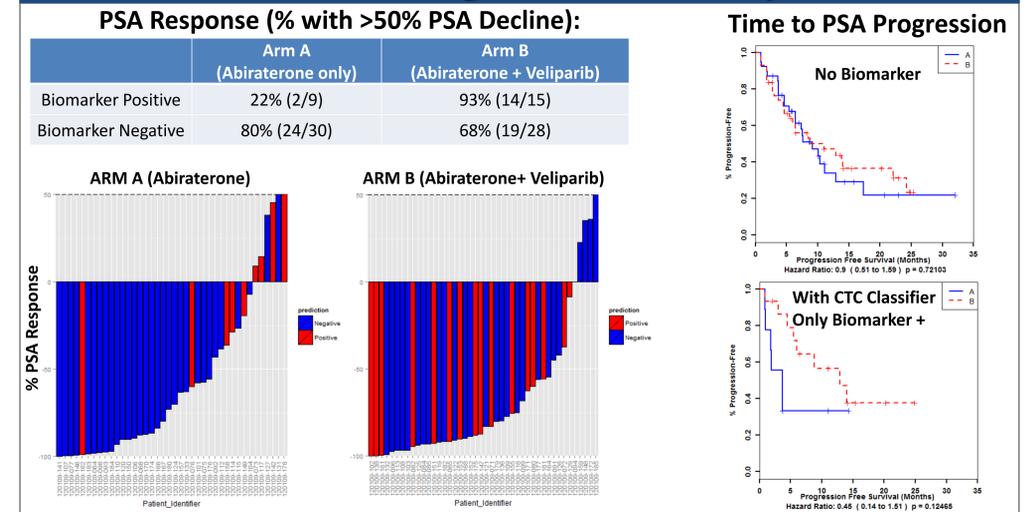LST (Genomic Instability)

210 CTCs from 27 patients were sequenced and generated a LST score (y axis), with high scores associating with genomic scars. Each dot represents a single CTC, with significant subclonal heterogeneity observed in multiple patients. Patients were analyzed for BRCA/ATM alterations from metastatic biopsy (MiOncoseq).
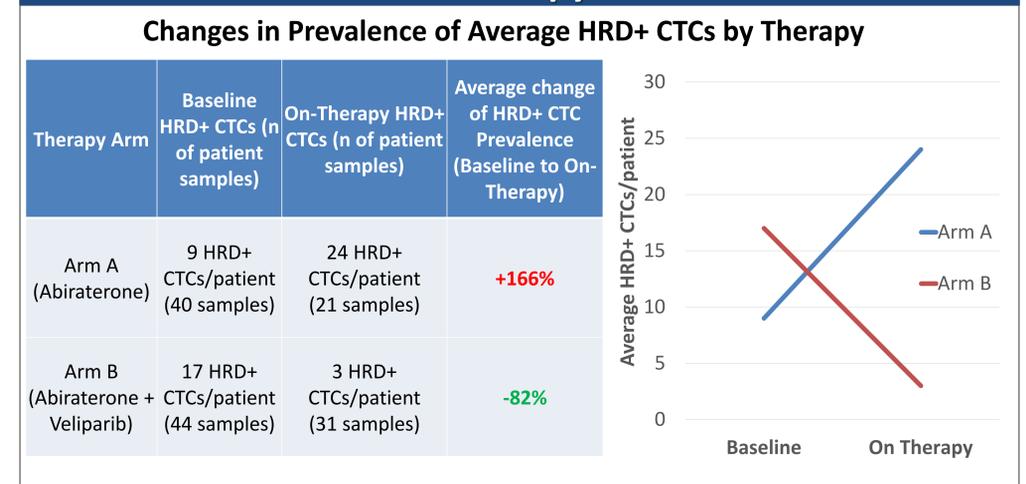
Patient Baseline Incidence of CTC Classifier in Cohort

	Arm A (Abiraterone only)	Arm B (Abiraterone + Veliparib)	Total
Biomarker Positive	23% (9/39)	35% (15/43)	29% (24/82)
Biomarker Negative	77% (30/39)	65% (28/43)	71% (58/82)

Patients with CTC Classifier Have Improved PSA Response and Time to Progression with Veliparib

PSA Response (% with >50% PSA Decline):

	Arm A (Abiraterone only)	Arm B (Abiraterone + Veliparib)
Biomarker Positive	22% (2/9)	93% (14/15)
Biomarker Negative	80% (24/30)	68% (19/28)

ARM A (Abiraterone) | ARM B (Abiraterone+ Veliparib)
% PSA Response — Patient_Identifier
prediction: Negative / Positive

Time to PSA Progression

No Biomarker
Hazard Ratio: 0.9 (0.51 to 1.59) p = 0.72103

With CTC Classifier Only Biomarker +
Hazard Ratio: 0.45 (0.14 to 1.51) p = 0.12465

HRD+ CTCs Increase or Decrease dependent upon Patient Therapy

Changes in Prevalence of Average HRD+ CTCs by Therapy

Therapy Arm	Baseline HRD+ CTCs (n of patient samples)	On-Therapy HRD+ CTCs (n of patient samples)	Average change of HRD+ CTC Prevalence (Baseline to On-Therapy)
Arm A (Abiraterone)	9 HRD+ CTCs/patient (40 samples)	24 HRD+ CTCs/patient (21 samples)	+166%
Arm B (Abiraterone + Veliparib)	17 HRD+ CTCs/patient (44 samples)	3 HRD+ CTCs/patient (31 samples)	-82%

Average HRD+ CTCs/patient — Baseline / On Therapy — Arm A / Arm B

Conclusions

- A previous CTC phenotypic algorithm (Scher et al.) trained on CTCs to identify high Genomic Instability (high LST), occurs more frequently in tissue confirmed BRCA/ATM mutant, but also in patients who do not have confirmed tissue HRD inactivating mutations.
- A novel biomarker obtained from a simple peripheral blood draw, utilizing CTC phenotypes to identify HRD+ and PARP resistance occurs in 29% of mCRPC 1st line patients.
- Patients with the PARP sensitive CTC biomarker and HRD+ CTCs have improved PSA ORR (93% vs. 22%) and trend to improved time to PSA progression (HR=0.45; p=0.12) with Abiraterone + Veleparib treatment compared to Abiraterone alone.
- The CTC HRD+ phenotype cell counts increase on average increase in patients receiving Abiraterone only, but on average decrease in patients receiving Abiraterone + Veliparib, supporting cellular PARPi sensitivity.
- Further development of single CTC phenotypic classifiers could help with patient selection, and monitoring of efficacy of novel HRD targeted therapies.