# CTC phenotype classifier identifies mCRPC patients with high genomic instability CTCs and predicts failure of Androgen Receptor signaling (AR Tx) and taxane systemic therapies

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## Background

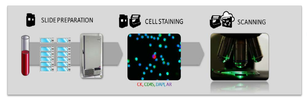
The reliable or accurate pretreatment prediction of a patient's response to AR Tx or Taxane chemotherapy is an unmet medical need, as some patients may respond to both classes of drugs, some to one but not the other, and others are resistant to both. Molecular profiling studies of mCRPCs suggest an association between high genomic instability (GI) and lack of response to either class of drugs.

Our overarching objectives are:

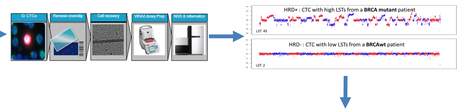
1) To develop blood based assays of circulating tumor cells (CTCs) to predict GI using phenotypic features from individual CTCs without next generation sequencing (NGS). And,
2) To evaluate the performance of the assay for the context of use for identifying patients for whom alternative approaches can be explored early avoiding the toxicity of ineffective standard of care therapies.

## Methods for CTC Detection; Phenotypic, Genomic Characterization

1) **Epic CTC Detection Platform**



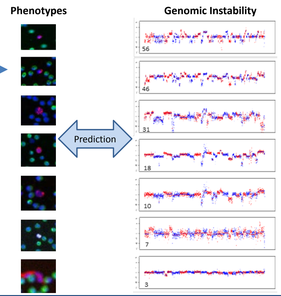

3) **Relocation and Capture of Single Cells for Genotyping and Genomic Instability**




2) **Single Cell Features**

| Protein Biomarker Features |
|---|
| CK cRatio (protein expression) |
| AR cRatio (protein expression) |
| **Digital Pathology Features** |
| Nuclear Area ($um^2$) |
| Cytoplasmic Area($um^2$) |
| Nuclear Convex Area ($um^2$) |
| Cytoplasmic Convex Area ($um^2$) |
| Nuclear Major Axis (um) |
| Cytoplasmic Major Axis (um) |
| Nuclear Minor Axis (um) |
| Cytoplasmic Minor Axis (um) |
| Nuclear Circularity |
| Cytoplasmic Circularity |
| Nuclear Solidity |
| Cytoplasmic Solidity |
| Nuclear Entropy |
| Nuclear to Cytoplasmic Convex Area Ratio |
| Nucleoli |
| CK Speckles |
| Nuclear Speckles |
| **Additional Categorical Variables** |
| CK Status (CK Positivity) |
| M1 Status (AR positivity) |
| CTC Cluster Status |

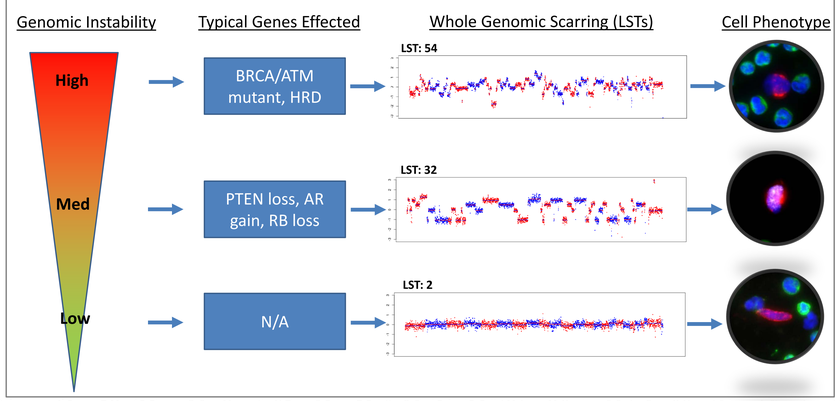
4) **Algorithm Development:**
Multivariate classifier to predict Genomic Instability from Phenotype




**Schematic of Epic CTC Platform CTC Enumeration, Morphology, and Biomarker Analyses Workflow:**

1) 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.
2) CTCs are then segmented within the DAPI, CK, and AR channels and ~20 nuclear and morphological features are extracted and classified.
3) Single CTCs are lysed, whole genome amplified, shotgun libraries constructed, and whole genome sequenced. Whole genome CNV categorizes 1Mb segments for amplifications or deletions. N of chromosomal breakpoints for regions >10Mb are scored as large scale transition (LST)[2].
4) In a training set of 597 single CTCs from 25 patients, 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 these models is a predicted LST (pLST) per CTC. Next, the models were cross-validated within the training cohort to assess accuracy using 2x2 thresholding. The final regression algorithm was then applied to rest of cohort not yet seen by the algorithm, and analyzed for clinical relevance by exploring associations to clinical outcomes on standard of care drugs.

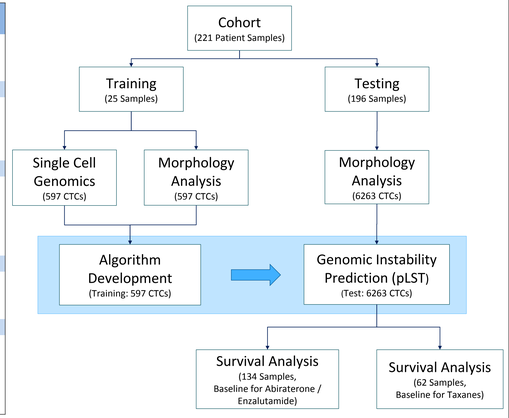
## Measuring Genomic Instability (Genotype to Phenotype)




**References:** 1) Popova T, Manié E, Rieunier G, Caux-Moncoutier V, Tirapo C, Dubois T, Delattre O, Sigal-Zafrani B, Bollet M, Longy M, Houdayer C, Sastre-Garau X, Vincent-Salomon A, Stoppa-Lyonnet D, Stern MH. Ploidy and large-scale genomicinstability consistently identify basal-like breast carcinomas with BRCA1/2inactivation. Cancer Res. 2012 Nov 1;72(21):5454-62. 2) Stephanie Greene, Jerry Lee, Mark Landers, Sandeep Sanga, Adam Jendrisak, Ryon Graf, Jessica Louw, Shannon Werner, Yipeng Wang, Ryan Dittamore, Dena Marrinucci. Single cell genomic profiling of circulating tumor cells (CTCs) from metastatic colorectal cancer (mCRC) identify tumor heterogeneity and rare somatic driver alterations. [abstract]. In: Proceedings of the AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics; 2015 Nov 5-9; Boston, MA. Philadelphia (PA): AACR; Mol Cancer Ther 2015;14(12 Suppl 2):Abstract nr A35.

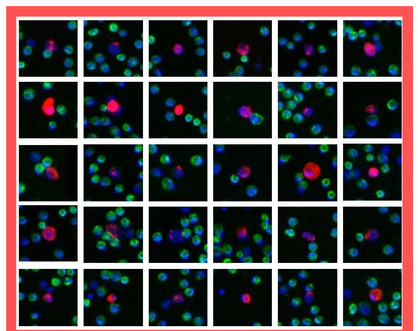
## Patient Demographics & Study Design

| Characteristic | No. (%) or Median (range) |
|---|---|
| Number of Baseline Samples (unique patients) | 221 (179) |
| Age, years | 68 (45 - 91) |
| **Primary Treatment** | |
| Prostatectomy | 100 (45%) |
| Radiation | 41 (19%) |
| Brachytherapy | 13 (6%) |
| None | 67 (30%) |
| **Hormone Therapies** | |
| 1 - 2 lines | 81 (37%) |
| 3 lines | 46 (21%) |
| ≥ 4 lines | 94 (43%) |
| Chemo-naïve | 136 (62%) |
| Chemo-exposed | 85 (38%) |
| **Metastatic Disease** | |
| Bone | 194 (88%) |
| Lymph Node | 149 (67%) |
| Visceral Mets | 76(35%) |
| **Laboratory Measures** | |
| PSA, ng/mL | 37.7 (0.10 – 3728.2) |
| Hgb, (g/dl) | 12.0 (7.0 – 15.0) |
| ALK, (unit/L) | 110 (25 – 2170) |
| LDH, (unit/L) | 222.5 (123 – 1293) |
| ALB, (g/dl) | 4.2 (3.1 – 4.9) |



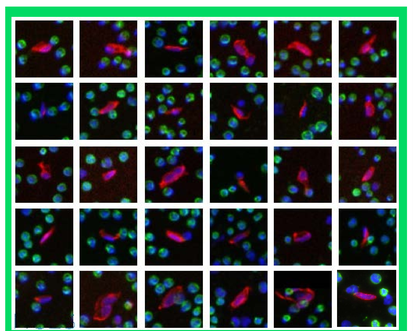

## High and Low Genomic Instability CTCs Identified by Distinct and Unique Phenotypic Features

Representative imagery from CTCs with high genomic instability (high LSTs) and low genomic instability (low LSTs) and key features associated with phenotypic classifier



High Genomic Instability (high LSTs)

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



Low Genomic Instability (low LSTs)

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

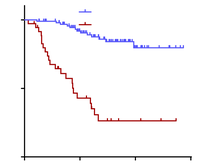
## Prevalence of Patients with Phenotypic Genomic Instability

| Biomarker | 1st Line | 2nd Line | 3rd+ Line | Total |
|---|---|---|---|---|
| ≥3 pLST+ CTC /mL | **23% (16)** | **24% (12)** | **40% (31)** | **30% (59)** |
| < 3 pLST+ CTC/mL | 77% (54) | 76% (37) | 60% (46) | 70% (137) |
| **Total (*n*)** | 70 | 49 | 77 | 196 |

## Patients with High Phenotypic Genomic Instability CTCs Have Inferior Survival (Univariate)

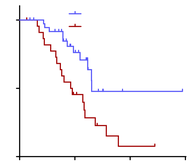
Kaplan-Meier estimations of overall survival are shown for all patient samples in separate test cohort scored by algorithm predicting presence of genomic instability from CTC phenotypic features. Patient samples drawn immediately prior to administration of standard of care drug class indicated, and comprises patients from all lines of therapy and various treatment histories.

**AR Tx (*n*=134)**



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

**Taxane (*n*=62)**



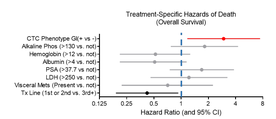
| | |
|---|---|
| Median Survival: | 9.4mo vs. 13mo |
| Hazard Ratio: | 2.79 |
| Log Rank p value: | 0.0050 |

## High Phenotypic Genomic Instability CTCs Are Prognostic for Poor Survival in Multivariate Model

Previous panel categorical classification of patients by biomarker status (high or low concentration of predicted high genomic instability CTCs) was incorporated into multivariate models of all factors statistically associated with overall survival ($p < 0.05$) as univariate features, as described in Scher et al 2016. Independent test cohort shown. Hazard Ratios and p-values represent adjusted, independent prediction of patient outcome by therapeutic class.
CTC phenotypic genomic instability was the most predictive biomarker for each drug class.

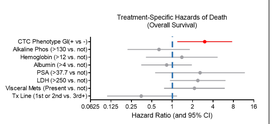
**AR Tx (*n*=134)**

| Pre-AR Tx Multivariate Feature | p value | HR | 95% CI low | 95% CI high |
|---|---|---|---|---|
| **Tx Line (1st or 2nd vs. 3rd+)** | **0.032** | **0.42** | **0.19** | **0.93** |
| Visceral Mets (Present vs. Not) | 0.56 | 0.71 | 0.22 | 2.25 |
| LDH (>250 vs. not) | 0.69 | 1.22 | 0.46 | 3.25 |
| PSA (>37.7 vs not) | 0.21 | 1.69 | 0.75 | 3.84 |
| Albumin (>4 vs. not) | 0.11 | 0.51 | 0.21 | 1.15 |
| Hemoglobin (>12 vs. not) | 0.15 | 0.52 | 0.21 | 1.26 |
| Alkaline Phos (>130 vs. not) | 0.17 | 1.82 | 0.77 | 4.27 |
| **CTC Phenotype GI (+ vs -)** | **0.022** | **2.95** | **1.17** | **7.47** |




**Taxane (*n*=62)**

| Pre-Taxane Multivariate Feature | p value | HR | 95% CI low | 95% CI high |
|---|---|---|---|---|
| Tx Line (1st or 2nd vs. 3rd+) | 0.091 | 0.35 | 0.11 | 1.18 |
| Visceral Mets (Present vs. Not) | 0.15 | 2.13 | 0.76 | 5.97 |
| LDH (>250 vs. not) | 0.072 | 2.39 | 0.93 | 6.12 |
| PSA (>37.7 vs not) | 0.22 | 2.58 | 0.56 | 11.8 |
| Albumin (>4 vs. not) | 0.67 | 0.83 | 0.35 | 1.95 |
| Hemoglobin (>12 vs. not) | 0.59 | 1.39 | 0.41 | 4.69 |
| Alkaline Phos (>130 vs. not) | 0.39 | 0.64 | 0.22 | 1.82 |
| **CTC Phenotype GI (+ vs -)** | **0.021** | **3.02** | **1.19** | **7.66** |




After adjustment for factors shown to have prognostic value in the cohort, the presence of ≥ 3 high pLST CTCs per mL remained the strongest factors in the models for overall survival on both AR Tx and Taxane.

## Conclusions

- An algorithm was developed that identifies CTCs with high genomic instability from their phenotypic features alone. Accuracy: 80%.
- A cut point of high genomic instability CTC/mL was found to maximize prognostication on SOC drugs. The overall prevalence of patients this biomarker(+) classification was 30% (59/196), and was associated with inferior OS times on AR Tx (HR=7.69, p<0.0001) and taxane therapy (HR=2.79, p=0.0050).
- High Phenotypic Genomic Instability helps identify patients who are resistant to the approved AR Tx and taxanes, for whom alternative approaches should be considered.
- This rapid blood imaging analysis will also help to screen patients for HRD directed therapies and to improve patient outcomes.

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