Glucocorticoid receptor (GR) expression in circulating tumor cells (CTCs) prognosticates poor overall survival (OS) for metastatic castration-resistant prostate cancer (mCRPC) patients (pts) treated with androgen receptor signaling inhibitors (ARSi)

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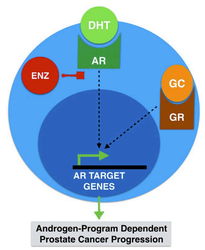
Background

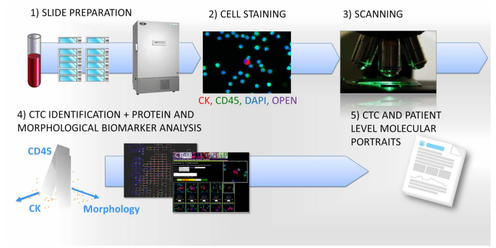
- GR upregulation in mCRPC is an alternate mechanism of resistance to androgen receptor signaling inhibitors (ARSi) such as enzalutamide (Enza) and abiraterone acetate (Abi).
- Pre-clinical studies implicate GR as a potential therapeutic target.
- We developed an assay as part of the Epic Sciences platform to assess GR protein expression on individual circulating tumor cells (CTCs).
- We sought to determine if the presence of CTCs with upregulated GR protein prior to initiation of either Abi or Enza represented an aggressive disease subset.

GR Promotes CRPC Resistance to Enzalutamide



Methods

- 54 mCRPC pt blood samples were collected prior to starting Abi (16) or Enza (38).
- The cohort was selected based on very favorable or very unfavorable PSA response by PCWG3 criteria.
- Blood samples were plated on microscope slides and every nucleated object imaged, with CTCs detected by a combination of: cytokeratin (CK) expression, intact nucleus, lack of CD45 (blood lineage) staining, and malignant morphology.

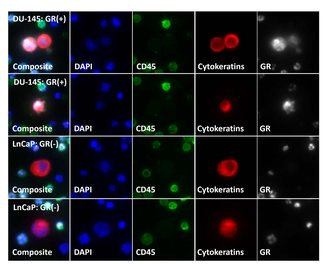


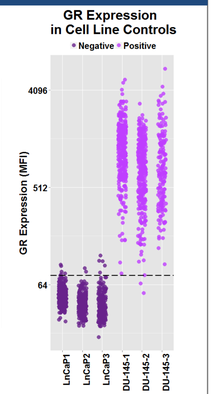
Schematic of Epic CTC Platform CTC enumeration, morphology, & biomarker workflow:

1) Nucleated cells from blood sample placed onto slides and stored in a -80°C biorepository
2) Slides stained with cytokeratin (CK), CD45, DAPI, GR
3) Slides scanned
4) CTC candidates detected by a multi-parametric digital pathology algorithm
5) Human reader confirmation of CTCs & quantitation of biomarker expression

Single-CTC GR Positivity Assessment

- Monoclonal antibody (clone D6H2L) specific to the glucocorticoid receptor (GR) C-terminal domain was evaluated on single cell line cells spiked into healthy donor blood and processed as pt samples.
- LnCaP (GR negative) and DU-145 (GR positive) prostate cancer cell lines were utilized.
- GR positivity was defined as mean fluorescent intensity (MFI) of 77.7, the 95th percentile of LnCaP GR expression.
- Due to co-expression of GR on WBCs, only CK+ CTCs could be assessed for GR expression.





Patient Demographics

Patient Characteristic	All Patients		
Number of Unique Patients	53		
Age, Years	70 (45 – 87)		
Primary Treatment			
Prostatectomy	27 (51%)		
Radiation	9 (17%)		
Brachytherapy	1 (2%)		
None	16 (30%)		
Sample Characteristic	**Pre-Enza Therapy**	**Pre-Abi Therapy**	**All Samples**
Number of Baseline Samples	38	16	54
Age, years	69 (45 - 87)	73 (48 - 86)	70 (45 - 87)
Treatment Decision*			
1st	23 (60%)	7 (44%)	30 (56%)
2nd	6 (16%)	5 (31%)	11 (20%)
3rd or later	9 (24%)	4 (25%)	13 (24%)
Prior Exposure to Life-Prolonging Therapies			
None	23 (61%)	7 (44%)	30 (56%)
AR only	5 (13%)	7 (44%)	12 (22%)
AR and Taxane ± other	10 (26%)	2 (13%)	12 (22%)
Chemotherapy Status			
Chemo-naïve	27 (71%)	14 (87.5%)	41 (76%)
Chemo-exposed	11 (29%)	2 (12.5%)	13 (24%)
Metastatic Disease			
Bone Only	14 (37%)	4 (25%)	18 (33%)
Lymph Node (LN) Only**	5 (13%)	4 (25%)	9 (17%)
Bone & LN**	14 (37%)	7 (44%)	21 (39%)
Bone & Visceral ± LN**	4 (10%)	1 (6%)	5 (9%)
Other Soft Tissue Only	1 (3%)	0	1 (2%)
Laboratory Measures Pre-Therapy: Median (range)			
PSA, ng/mL	34.8 (0.51 – 1516.11)	30.0 (8.35 – 1479.88)	33.6 (0.51 – 1516.11)
Hgb, (g/dl)	12.3 (7.0 – 14.4)	12.3 (10.0 – 14.9)	12.3 (7.0 – 14.9)
ALK, (unit/L)	100 (49 – 2170)	103 (59 – 539)	100 (49 – 2170)
LDH, (unit/L)	220 (139 – 1293)	228 (169 – 398)	220 (139 – 1293)
ALB, (g/dl)	4.2 (3.3 – 4.6)	4.2 (3.8 – 4.6)	4.2 (3.3 – 4.6)

*only includes SOC life-prolonging therapies and experimental therapies patient was exposed to after standard ADT and development of mCRPC disease and prior to initiation on the baseline therapy
**includes patients with other soft tissue disease

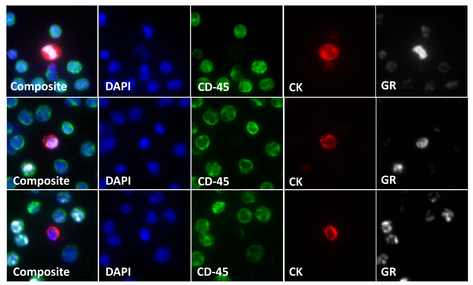
Pts with samples drawn for Epic CTC processing
(Unique pts n = 82, Samples n = 87)

→ **Excluded (not mCRPC or not a baseline sample for Abi or Enza)**
(Unique pts n = 29, Samples n = 33)

mCRPC Abi/Enza Baseline Samples Assessed for GR Testing, CTC Enumeration, and GR Expression
(Unique pts n = 53, Samples n = 54)

Survival Analyses:
(Unique pts n=53, Samples n=54)
Pts assessed for OS based on CTC enumeration and presence of GR(+) CTC(s)

Representative Images of GR CTCs from CRPC Patients

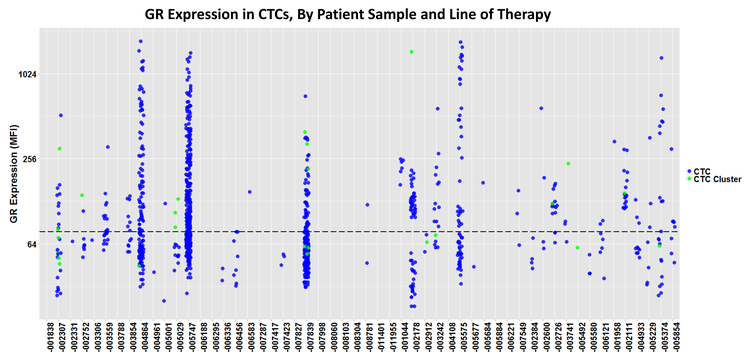


Positive (>77.7)
GR Expression: 1273.03

Positive (>77.7)
GR Expression: 341.00

Negative (<77.7)
GR Expression: 33.00

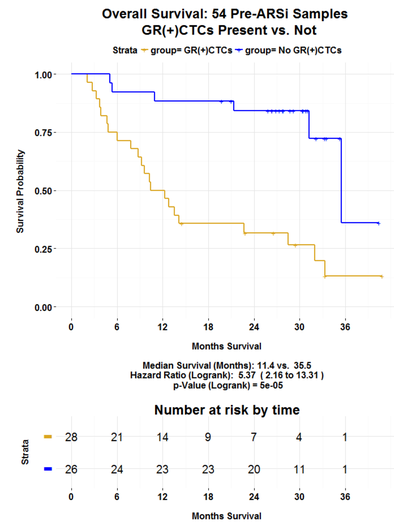
GR Protein Expression on CTCs is Prevalent Across Lines of Therapy



GR+, CK+ CTC(s) Prognosticate Worse Outcome on ARSi Than for Patients Without GR+, CK+ CTC(s)

Univariate Analysis

- The overall survival of pts with GR(+) CTCs (gold curve) prior to ARSi was compared to pts with either only GR(-) CTCs or no CTCs (blue curve).



Multivariate Analysis

- The cohort contains pts with 0 CTCs, only GR(-) CTCs, and patients who have GR(+) CTCs.
- The presence of any CTCs was compared to the presence of GR(+) CTCs, both prognostic markers as univariates.

Covariate	Multivariate HR (95% CI)	Multivariate *p* Value
Presence of (any) CTCs	1.58 (0.31 – 7.90)	0.57
Presence of GR(+) CTCs	4.16 (1.23 – 14.0)	0.02

Conclusions

- GR protein upregulation in CTCs can be detected in a majority of mCRPC samples tested (28 of 54, 52%).
- In this cohort, the presence of GR(+) CTCs was a stronger negative prognostic marker for overall survival than the presence of only GR(-) CTCs or no CTCs.
- Survival analyses are consistent with the hypothesis that GR expression portends more aggressive disease with greater ability to resist AR signaling inhibition, as assayed by tumor cells in circulation.
- Detection of GR in CTCs from mCRPC patients may be a useful biomarker to guide GR-directed therapies.

Support: MSKCC SPORE in Prostate Cancer (P50 CA92629), the Department of Defense Prostate Cancer Research Program (PC051382), NIH/NCI Cancer Center Support Grant P30 CA0098748, The Prostate Cancer Foundation, and the David H. Koch Fund for Prostate Cancer Research.