Changes in CTC burden and phenotypes in mCRPC patients (pts) receiving alpharadin (Ra-223) as single agent or in combination with other therapeutics (Tx)

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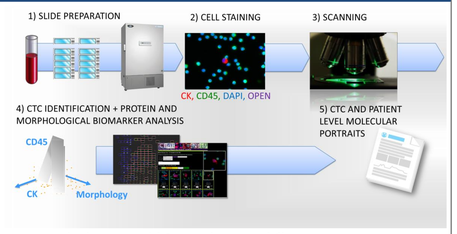
Background

- In unselected patient populations, Ra-223 (Xofigo) demonstrated an improvement in overall survival (OS). However, a lack of predictive and pharmacodynamic (PD) biomarkers to inform patient selection and confirm efficacy remain an unmet medical need.
- Ra-223 is being studied in combination with androgen receptor signaling inhibitors (ARSi), abiraterone, enzalutamide or taxane chemotherapy, further confounding the identification and validation of predictive and PD biomarkers.
- Pre-clinical data supports that Ra-223 may induce and sensitize tumors to DNA damaging agents and/or checkpoint inhibitors.
- We sought to evaluate the relationship between CTC counts and phenotypic changes for both single agent Ra-223 and combined Ra-223 + ARSi from pre-treatment and on-therapy blood draws and patient outcomes.

Methods for CTC Detection

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, and 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, Androgen Receptor
3) Slides scanned
4) CTC candidates detected by a multi-parametric digital pathology algorithm
5) Human reader confirmation of CTCs & quantitation of biomarker expression

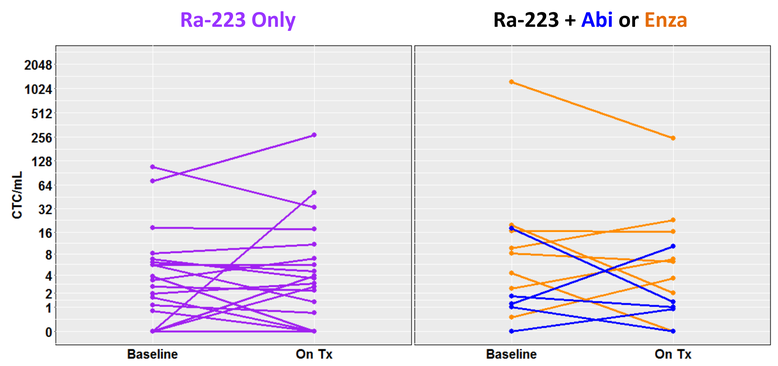
Patient Demographics

- This cohort represents a retrospective analysis of prospectively enrolled and banked samples (see above) and exploratory analyses
- **Sample inclusion criteria:** pts must have one draw before initiation of Ra-223, either as a single agent or in combination with abiraterone or enzalutamide, and one draw taken during therapy.
- 34 pts contributed two samples each
- 20 pts received Ra-223 alone
- 14 pts received a combination of Ra-223 and abiraterone or enzalutamide
- One patient excluded: abiraterone was added to Ra-223 after therapy initiation
- As of 25 May 2017, there are 22 death events among the 33 pts

Patient Characteristic	All Patients
Number of Unique Patients	34
Age, Years	72 (48 - 89)
Primary Treatment	
Prostatectomy	13 (38%)
Radiation	9 (27%)
Brachytherapy	2 (6%)
None	10 (29%)
Sample Characteristic	
Total Number of Samples	68
Baseline Samples	34
On-treatment Samples	34
Treatment Decision *	
1st	6 (18%)
2nd	9 (26%)
3rd or later	19 (56%)
Prior Exposure to Life-Prolonging Therapies	
None	4 (12%)
AR only	14 (41%)
AR AND Taxane ± other	16 (47%)
Chemotherapy Status	
Chemo-naïve	18 (53%)
Chemo-exposed	16 (47%)
Metastatic Disease	
Bone Only*	14 (41%)
Bone & LN*	18 (53%)
Bone, LN & Other Soft Tissue	1 (3%)
Bone & Visceral ± LN*	1 (3%)
Laboratory Measures Pre-Therapy: Median (range)	
PSA, ng/mL	42.67 (1.38 – 941.22)
Hgb, (g/dl)	12.1 (10.0 – 14.3)
ALK, (unit/L)	132 (38 – 1208)
LDH, (unit/L)	212 (135 – 895)
ALB, (g/dl)	4.15 (3.6 – 4.7)

* Includes pts with local recurrence

CTC Enumeration Changes on Ra-223 Single Agent and Combination Therapy



35% (7/20) pts had CTC gains
15% (3/20) pts had no change
50% (10/20) pts had CTC drops

38% (5/13) pts had CTC gains

62% (8/13) pts had CTC drops

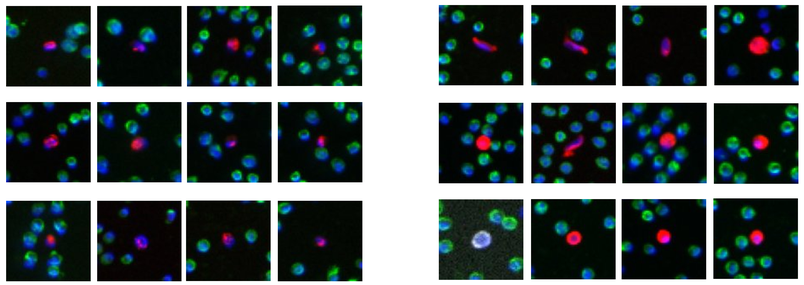
CTC Phenotypes Change During Ra-223 Single Agent Therapy

Phenotypes More Common in Baseline than On Tx Samples

- High Nuclear/Cytoplasmic Area Ratio
- Smaller Cytoplasmic Area

Phenotypes More Common in On Tx than Baseline Samples

- Low Nuclear/Cytoplasmic Area Ratio
- Larger Cytoplasmic Area
- Smaller Nuclear Area



Frequency of pts with CTC phenotype changes:

	% of pts w. P1	% of pts w. P2
Rise	10%	**30%**
Decline	**45%**	15%
Always Zero	45%	55%

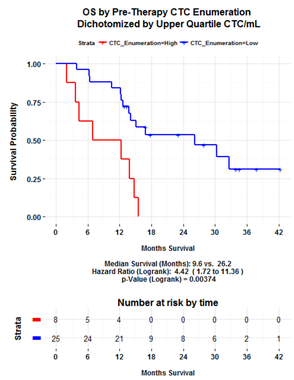
P1: Phenotypes More Common in Baseline than On Tx Samples

P2: Phenotypes More Common in On Tx than Baseline Samples

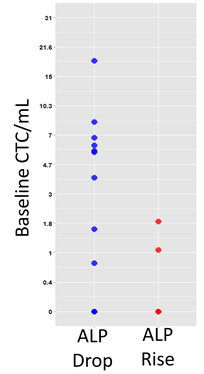
Baseline CTC Counts are Prognostic on Ra-223 Tx

Baseline Continuous Variable	p-value
ALK (log)	0.00059
LDH (log)	0.0025
PSA (log)	0.0014
HGB	0.15
ALB	0.78
Drug (single agent vs. combination)	0.48
CTC Baseline Continuous Variable	**p-value**
CTC (log)	0.00016
CTC On-Therapy Continuous Variable	**p-value**
CTC (log)	0.00053

- Cohort: Ra-223 single agent and Ra-223 + ARSi (*n* = 33, 22 death events)
- The relationship between overall survival and pre-clinical features was evaluated with univariate Cox models.



Combination of Baseline CTC Counts and Alkaline Phosphatase (ALP) Changes Potentially Improve Ra-223 Prognostics



Category	Events	Average Alive Months
ALP Drop, Low CTC (<3/mL)	4 Alive, 1 Deceased (20% Deceased)	25
ALP Drop, High CTC (>3/mL)	2 Alive, 5 Deceased (71% Deceased)	17
ALP Rise, High CTC (>3)	1 Alive, 3 Deceased (75% Deceased)	13

- Cohort: Ra-223 single agent (***n* = 20**).
- ALP Drop and Rise were determined by comparing baseline ALP level with 12 week follow up (Sartor *et al.* 2017 Annals of Oncology).
- pts with both ALP drop and low baseline CTC counts benefitted the most from Ra-223 Tx

Conclusions

- CTCs changes occur in pts receiving single agent or combination Ra-223 therapy, supportive that characterization of CTCs may reflect changes to the bone compartment due to Ra-223
- CTCs phenotypic changes occur in pts treated with Ra-223. Biological characterization of the specific cell types can potentially provide insights into treatment sensitivity
- Measurement of baseline and on-therapy CTC counts and phenotypes are being evaluated in larger cohorts to further develop predictive and PD biomarkers in context to Ra-223 Tx

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