Characteristics of *de novo* Resistance to Androgen Targeting Therapeutics (AR Tx) through Circulating Tumor Cells (CTCs) analyses in metastatic Castration Resistant Prostate Cancer (mCRPC) patients

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Richard Bambury[1,2], Mark Landers[3], Jessica Louw[3], Adam Jendrisak[3], Daniel Danila[1,2], Dana Rathkopf[1,2], Zaina Arslan[1], Nicole Schreiber[1], Rachel Krupa[3], Dena Marrinucci[3], Ryan Dittamore[3], Howard I. Scher[1,2]

1. Sidney Kimmel Center for Prostate and Urologic Cancers, Memorial Sloan-Kettering Cancer Center, New York, NY 2. Department of Medicine, Weill Cornell Medical College, New York, NY 3. Epic Sciences, La Jolla, CA

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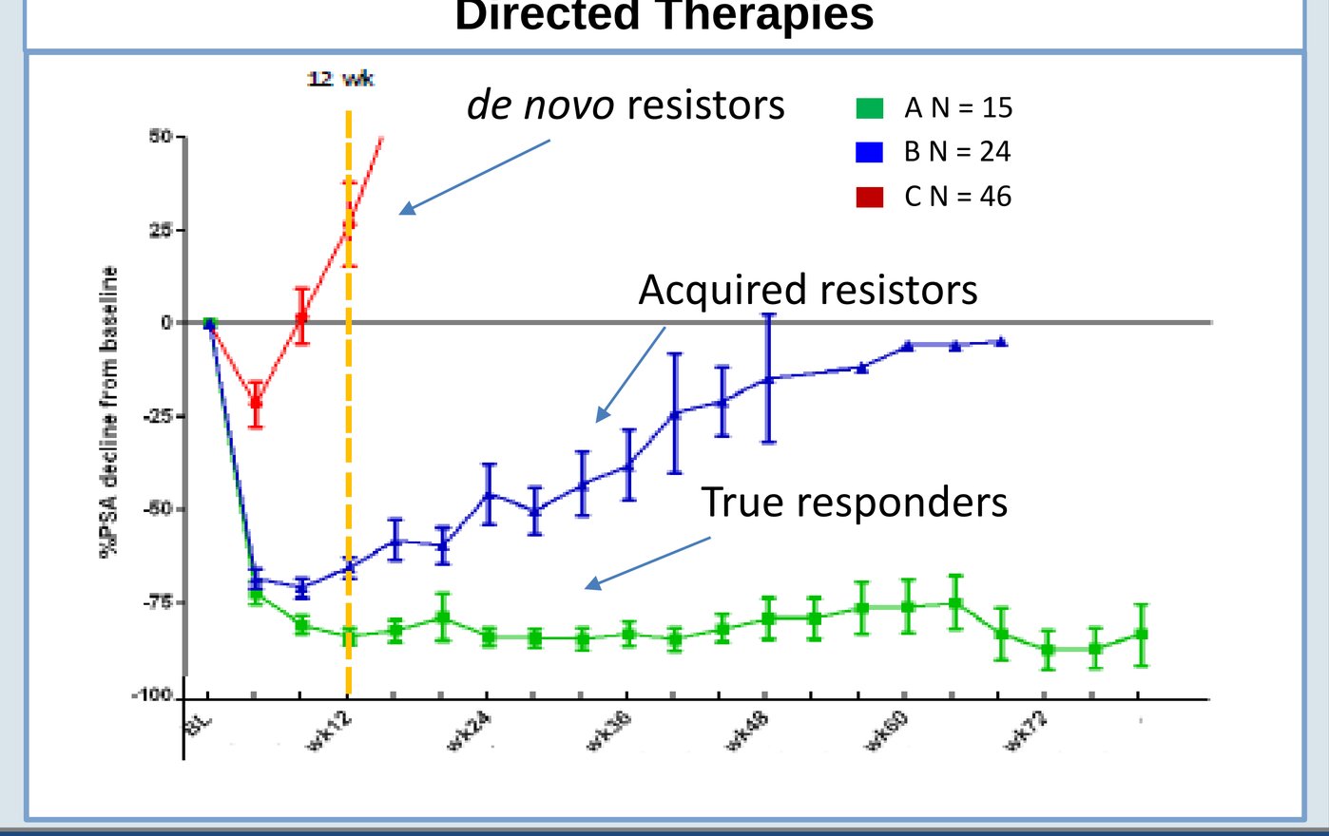
Background

- Novel, life extending therapies targeting the Androgen Receptor (AR) are now part of the standard of care in mCRPC.
- Response to these therapies is heterogeneous across patients.
- Prospective identification of patients with tumors that harbor *de novo* resistance to AR Tx could reduce morbidity and enable early exploration of alternative approaches.
- We examined total CTCs, specific CTC subpopulations and CTC AR protein expression from blood samples taken prior to Abiraterone Acetate + Prednisone (A), Enzalutamide (E) or taxane (T).
- Results were correlated with response patterns shown in Figure A.

Figure A. Patterns of PSA Changes After AR Signaling Directed Therapies



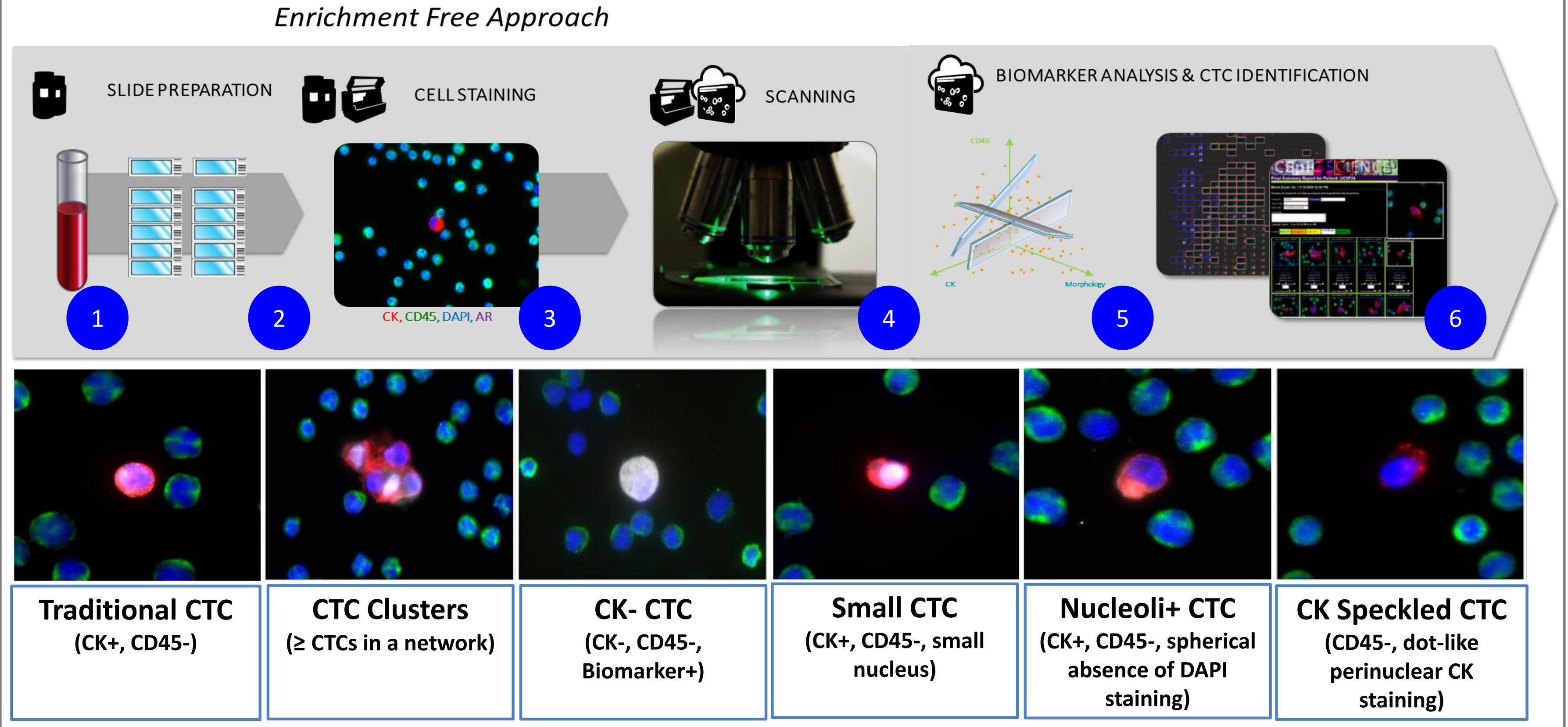
Methods

- 85 blood samples were collected from mCRPC pts immediately prior to treatment A, E or T. Patients on A, E or T were classified according to Figure A as:
- Responders (n=39) which included:
  - true response (n=15)
  - acquired resistance (n=24).
- Non responder (n=46): *de novo* resistance
- Baseline samples were processed utilizing the Epic Sciences platform:

Figure B: Schematic of Epic's CTC collection and detection process:

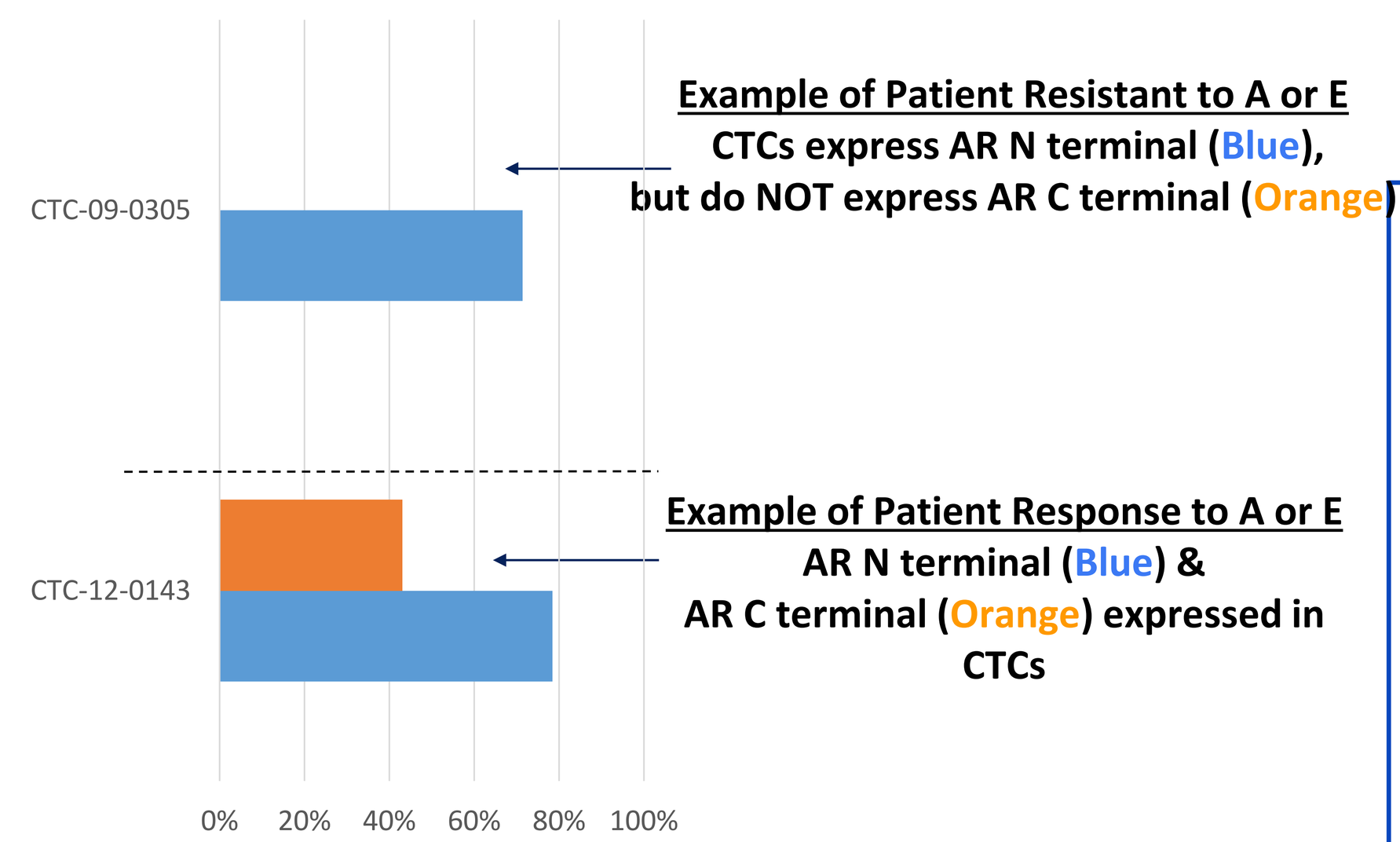
1) Blood lysed, nucleated cells from blood sample placed onto slides
2) Slides stored in -80C biorepository
3) Slides stained with CK, CD45, DAPI and AR
4) Slides scanned
5) Multi-parametric digital pathology algorithms run
6) Software and human reader confirmation of CTCs & quantitation of biomarker expression

Enrichment Free Approach



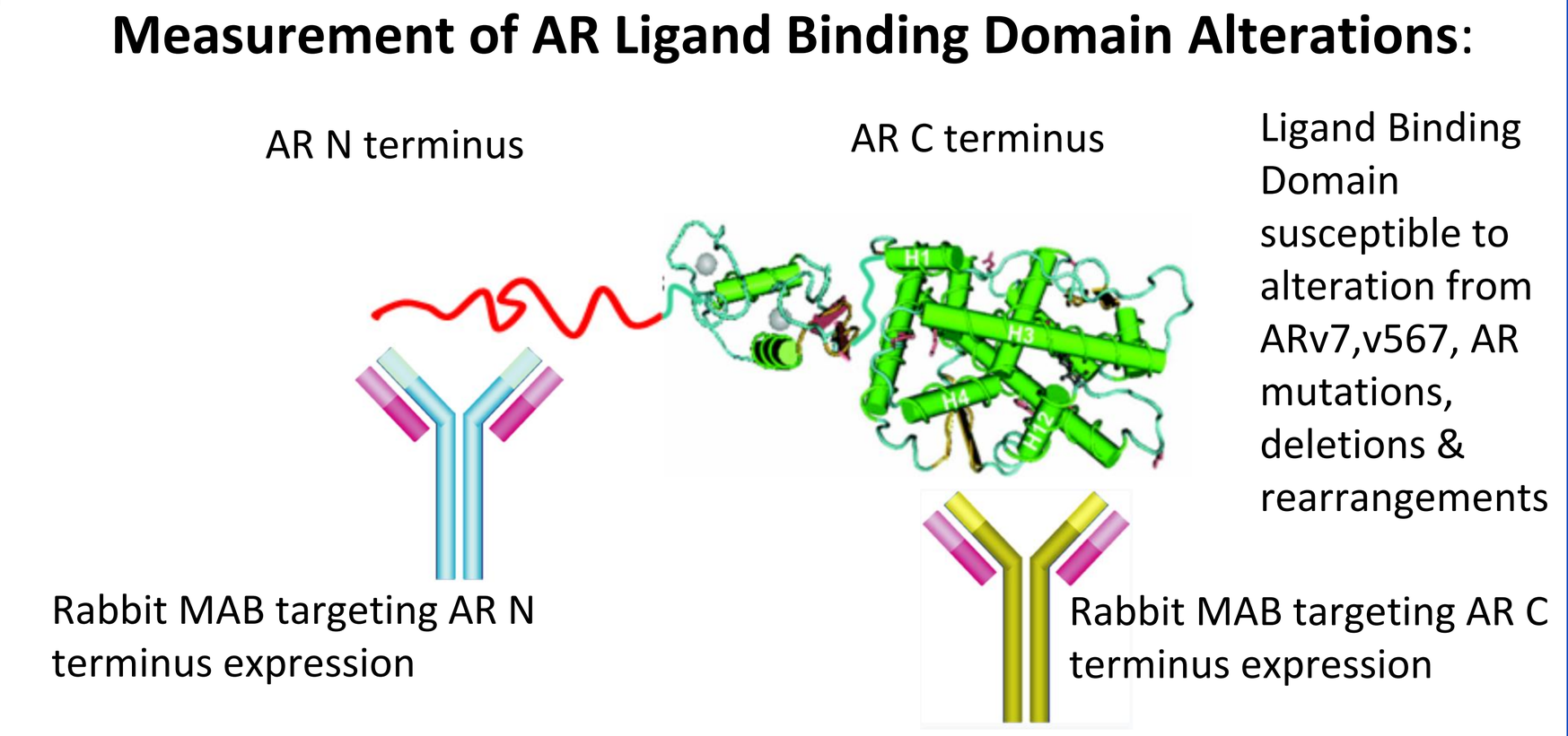
Measurement of AR Ligand Binding Domain Alterations

Figure C. Example of CTC AR N terminal to AR C terminal protein expression



CTCs were examined for AR Ligand Binding Domain (LBD) alterations utilizing a 2 target assay directed at AR N terminal protein expression and AR C terminal protein expression

Measurement of AR Ligand Binding Domain Alterations:



Study Population

Characteristic	No. (%) or Median (range)			
	All Patients	Responders		Resistors
		true responder	acquired resistance	*de novo*
Number of patients	85	15	24	46
Age, years	70 (48-91)	67 (48-82)	73 (52-91)	69 (48-86)
Primary Treatment				
Prostatectomy	35 (46%)	9 (82%)	11 (52%)	15 (34%)
Radiation	21 (28%)	1 (9%)	4 (19%)	16 (36%)
None	20 (26%)	1 (9%)	6 (29%)	13 (30%)
Metastatic Disease				
Bone	70 (92%)	9 (82%)	21 (100%)	39 (89%)
Lymph Node	48 (63%)	6 (55%)	11 (52%)	31 (70%)
Liver	5 (7%)	1 (9%)	0 (0%)	4 (9%)
Lung	6 (8%)	1 (9%)	3 (14%)	2 (5%)
Other Soft Tissue	3 (4%)	1 (9%)	0 (0%)	2 (5%)
Laboratory Measures				
PSA, ng/mL	53.32 (0.71-2589.9)	22.82 (10.77-1322.85)	28.59 (0.71-1774.49)	85.14 (7.94-2589.9)
Hgb, (g/dl)	11.6 (7.2-15.0)	12.0 (10.6-13.4)	12.0 (8.4-15.0)	11.0 (7.2-14.4)
ALK, (unit/L)	108 (42-952)	82 (42-190)	109 (51-857)	142 (49-952)
LDH, (unit/L)	237 (142-976)	215 (156-398)	236 (142-479)	242 (163-976)
ALB, (g/dl)	4.2 (3.3-4.9)	4.3 (4.1-4.9)	4.2 (3.6-4.9)	4.1 (3.3-4.7)
CTC, (cells/7.5mL)	6 (0 - >200)	4 (0-160)	1 (0 - >200)	14 (0 - >200)

Previous Patient Therapy

A or E Baseline Blood Draw (n=47)

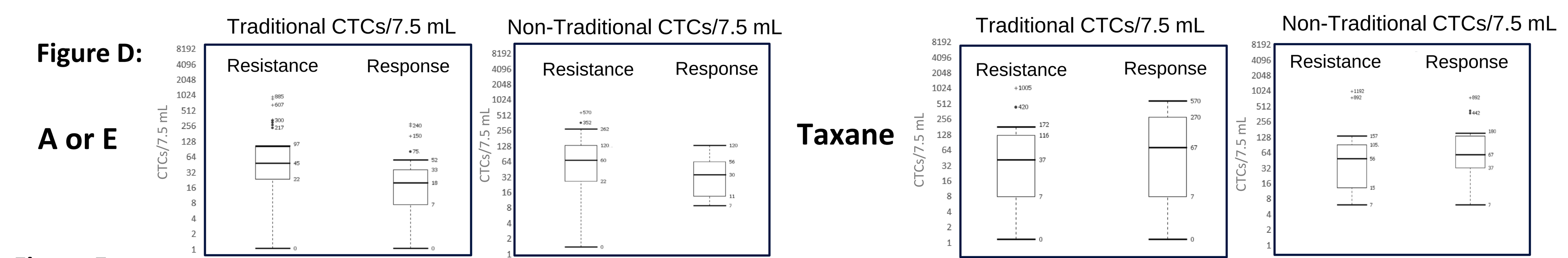
- No Previous AR TX (1st Line) n= 16
- Previous AR Tx (A or E) (2nd Line) n= 14
- Previous T (2nd Line) n= 2
- Previous AR Tx & T (3rd Line) n= 15

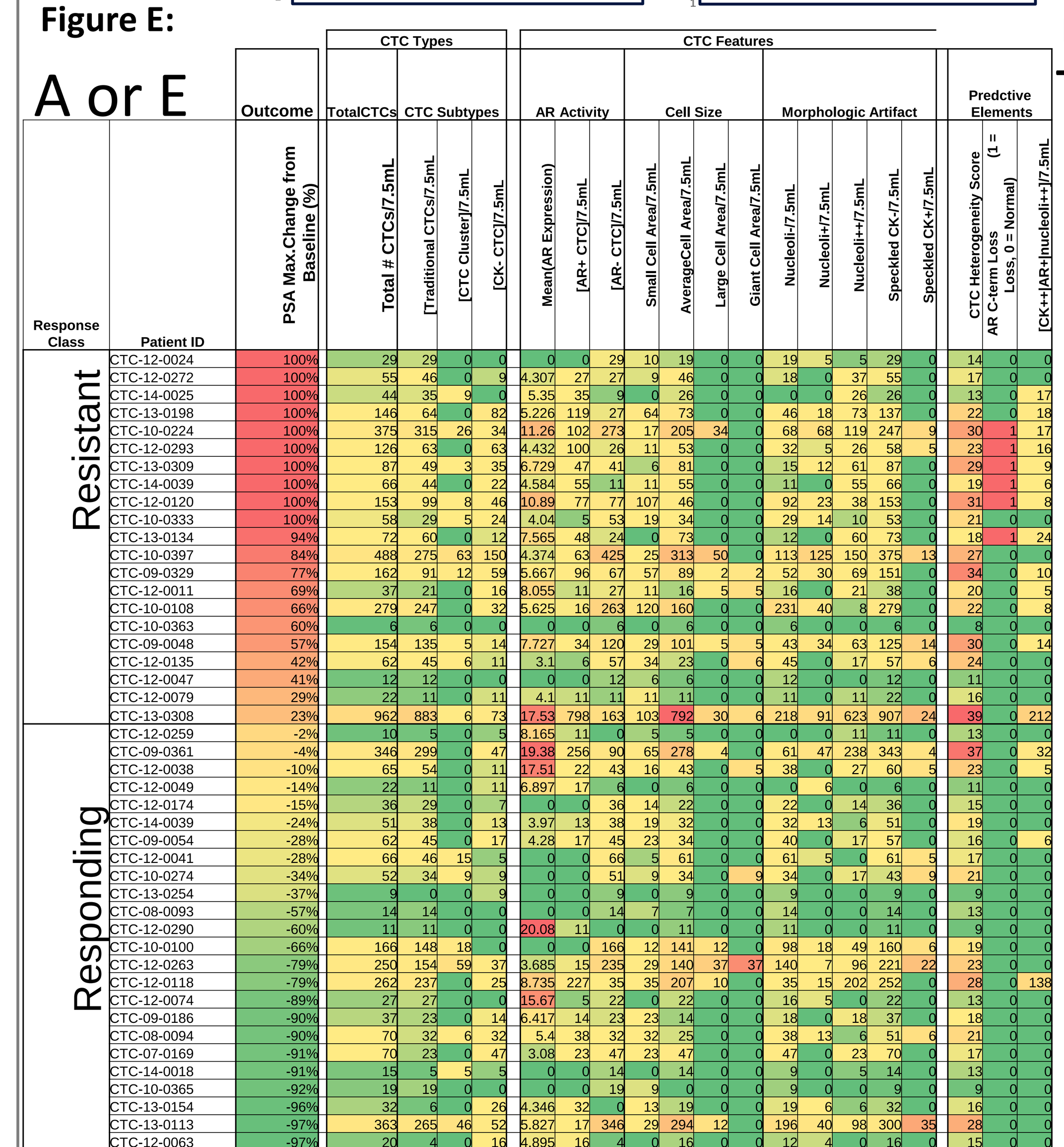
T Baseline Blood Draw (n=38)

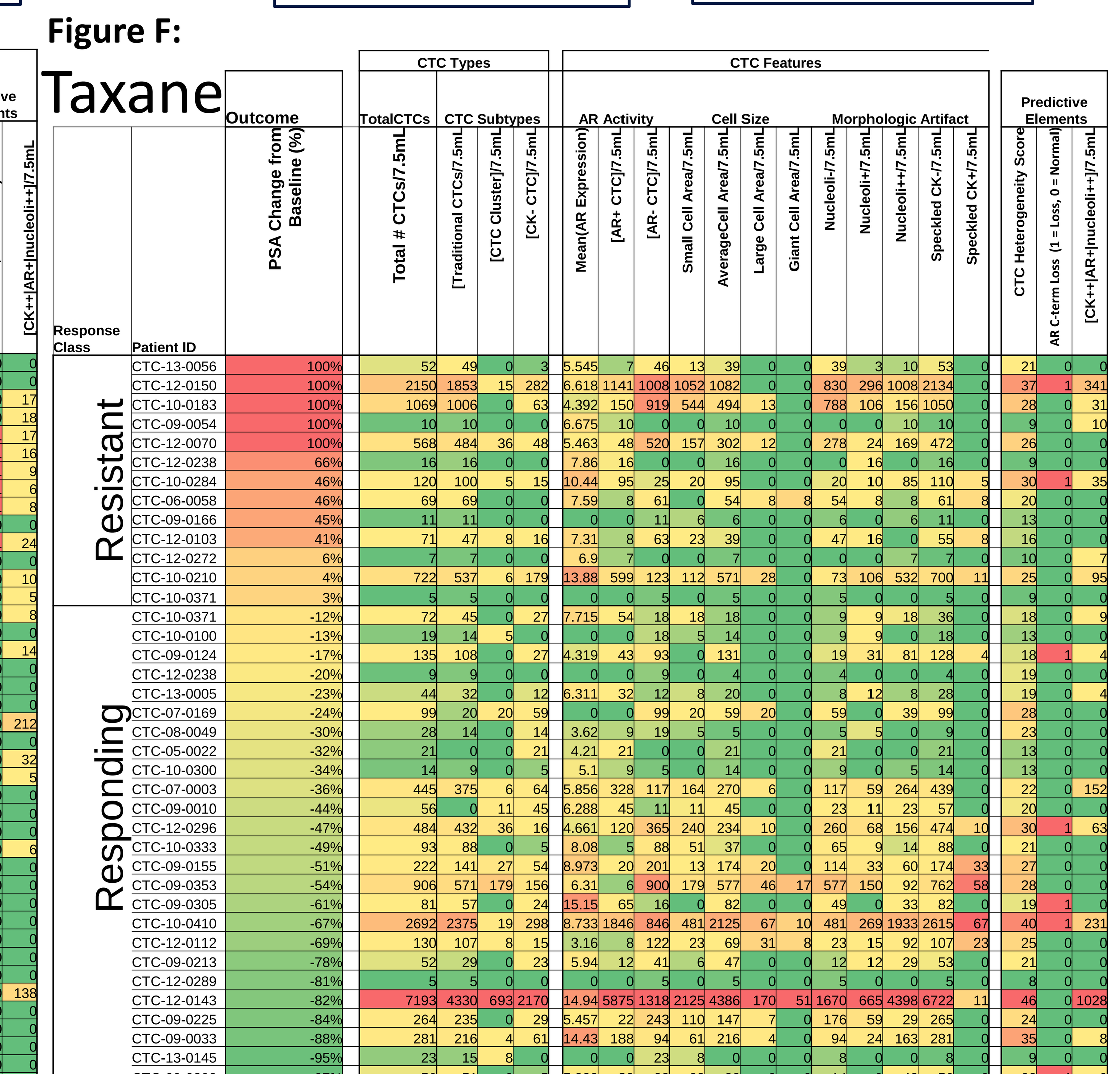
- No Previous AR TX (1st Line) n=3
- Previous AR Tx (A or E) (2nd Line) n= 7
- Previous A & E (3rd Line) n= 7
- Previous AR Tx & T (3rd Line) n= 21

Heterogeneity of CTC Characteristics by Response

Figure D: The boxplots below represent the number of traditional CTCs, and non-traditional CTCs per 7.5 mL vs. response to A or E (left) or Taxane (right). The box perimeter defines the upper and lower quartiles, the median value is the marker within the box. The whiskers represent 1.5 IQR of the observed values.







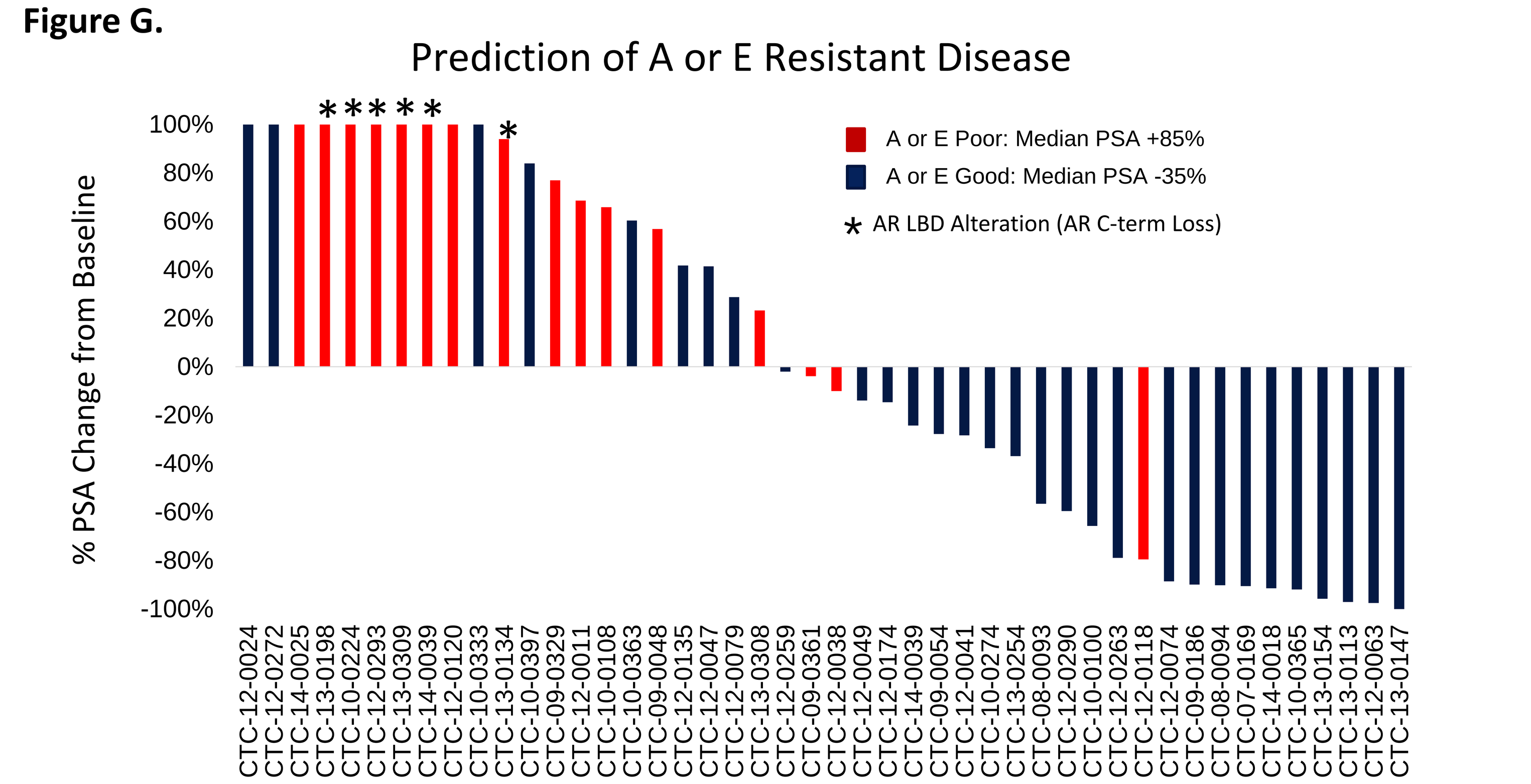
The heat map above lists the patients screened prior to 1st, 2nd or 3rd line A or E (**Figure E**), or taxane (**Figure F**) grouped by response vs. the relative abundance of specific CTC characteristics. CTC features are not mutually exclusive and may not represent unique events. Values displayed are reported in CTC/mL for each phenotype (except for Average AR+ /CTC) and are color coded to reflect relative abundance (high=red, mid=yellow, low=green) across all patients.

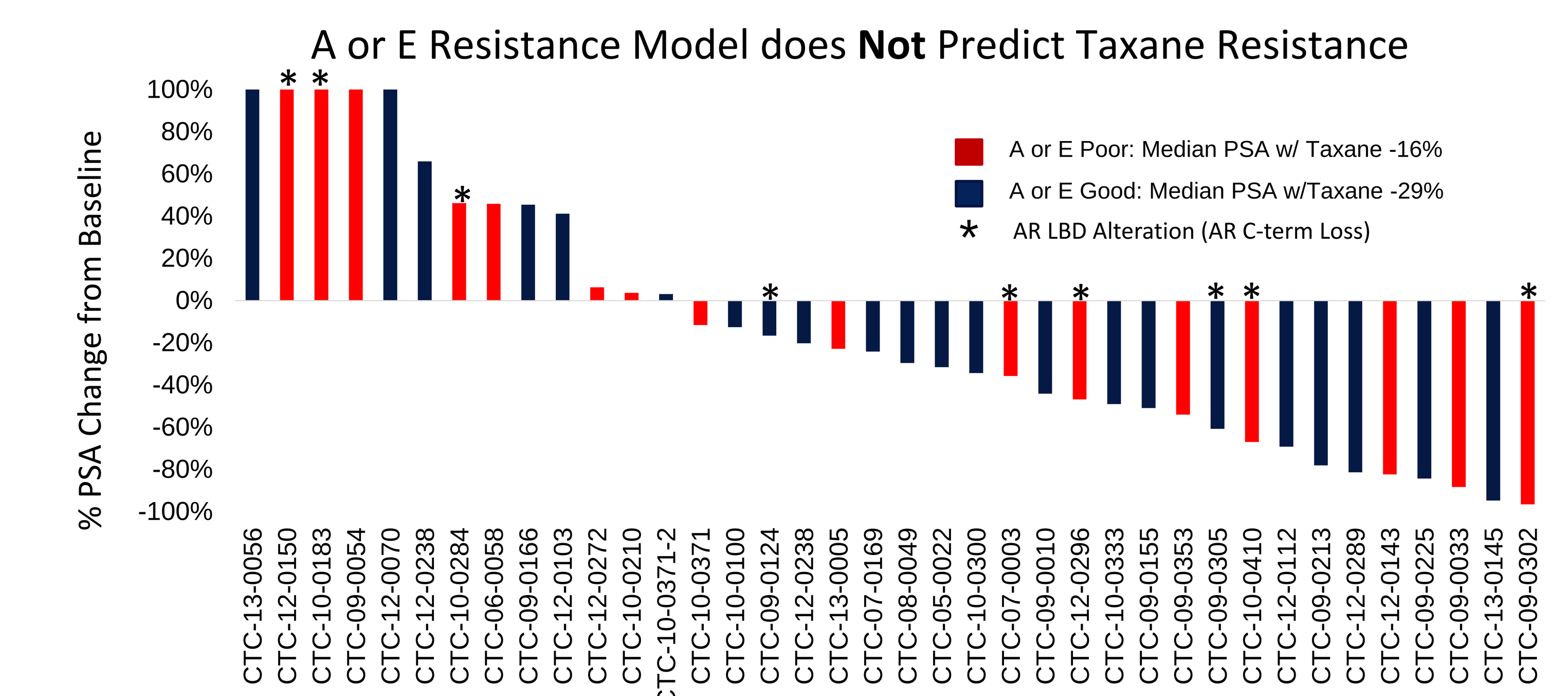
CTC Characterization Predicts AR Tx *de novo* Resistance

CTC Biomarkers Predict A or E Resistant Disease from Baseline Blood Samples		
Biomarker	**Odds Ratio**	**p-value**
CTC Heterogeneity	4.2	0.05
AR C-terminal loss	Inf.	0.004
CK++/AR+/Nucleoli++	8.531	0.002
Multivariate biomarker	11.9	0.001

Figure G. The table to the left compares both univariate and multivariate models for the prediction of AR Tx response using molecular and morphological variables. The waterfall plots below depict the relationship between AR Tx resistance prediction and % Max decrease PSA for patients treated with AR Tx (top) and taxane (bottom).

Figure G.





Conclusions

1. **CTC/7.5 mL or non-traditional CTC/7.5 mL frequency does not predict Abiraterone, Enzalutamide or Taxane resistance**
2. **Sensitivity to Abiraterone or Enzalutamide was assessable from baseline blood draw through the single cell CTC measurement of:**
   1. **CTC Heterogeneity**
   2. **Frequency of cytokeratin positive, AR N-terminal positive CTCs with prominent nucleoli morphology**
   3. **AR C-terminal loss**
3. **The CTC profile of sensitivity to Abiraterone and Enzalutamide resistant disease did not predict sensitivity to Taxane therapy, suggesting that the models may be useful to guide treatment selection.**
4. **Prospective validation of the predictive signatures in dedicated trials is planned.**

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