

# PS18-07: Differential odds of response in ILC versus IDC correlate with changes in the TIME in a phase II trial of pre-operative fulvestrant with or without enzalutamide



Anthony D. Elias<sup>1</sup>, Alyse W. Staley<sup>2,3</sup>, Monica Fornier<sup>4</sup>, Gregory A. Vidal<sup>5</sup>, Vida Alami<sup>6</sup>, Sharon Sams<sup>6</sup>, Nicole S. Spoelstra<sup>6</sup>, Andrew Goodspeed<sup>7,8</sup>, Peter Kabos<sup>1</sup>, Jennifer R. Diamond<sup>1</sup>, Elena Shagisultanova<sup>1</sup>, Rosa I Gallagher<sup>9</sup>, Julia Wulfkuhle<sup>9</sup>, Emanuel Petricoin<sup>9</sup>, Kathryn Zolman<sup>6</sup>, Tessa McSpadden<sup>10</sup>, Christian Rickert<sup>11</sup>, Kimberly R. Jordon<sup>11</sup>, Jill E. Slansky<sup>11</sup>, Virginia F. Borges<sup>1</sup>, Dexiang Gao<sup>2,3</sup>, Jennifer K. Richer<sup>6</sup>.

<sup>1</sup>Department of Medicine/Medical Oncology, University of Colorado, Anschutz Medical Campus, Aurora, Colorado, USA. <sup>2</sup>Department of Pediatrics, University of Colorado, Anschutz Medical Campus, Aurora, Colorado, USA. <sup>3</sup>University of Colorado Cancer Center, Biostatistics and Bioinformatics Shared Resource, Aurora, Colorado, USA. <sup>4</sup>Department of Medical Oncology, Memorial Sloan Kettering Cancer Center, New York, New York, USA. <sup>5</sup>West Cancer Center and Research Institute and Department of Medicine, University of Tennessee Health Sciences Center, Tennessee, USA. <sup>6</sup>Department of Pathology, University of Colorado, Anschutz Medical Campus, Aurora, Colorado, USA. <sup>7</sup>University of Colorado Cancer Center, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA. <sup>8</sup>Department of Biomedical Informatics, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA. <sup>9</sup>Center for Applied Proteomics and Molecular Medicine, George Mason University, Fairfax, Virginia, USA. <sup>10</sup>University of Colorado Cancer Center, Oncology Clinical Research Support Team, Aurora, Colorado, USA. <sup>11</sup>Department of Immunology and Microbiology, University of Colorado, Anschutz Medical Campus, Aurora, Colorado, USA.

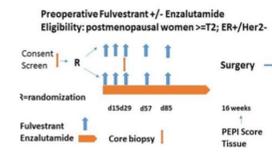
## Introduction

While over 90% of estrogen receptor positive (ER+) breast cancers (BC) express androgen receptors (AR), the role of AR and whether it can serve as a therapeutic target, remains contentious. In a randomized phase II trial for women with ER+/HER2- primary BC T2 or greater, neoadjuvant fulvestrant (Fulv) alone or in combination with the anti-androgen enzalutamide (Combo) was given for 4 months prior to surgery. A total of 59 patients were evaluable: 33 on Combo and 26 on Fulv. Tumor biopsies were obtained at study entry (baseline), after 4 weeks on therapy (W5), and at surgery. Laboratory analyses from FFPE sections of tumor included immunohistochemistry (IHC) for the steroid hormone receptors ER/PR/AR/GR and Ki67, evaluation of gene expression and multiplex immunofluorescence for immune cells on FFPE tumor tissues, and frozen tumor was utilized for reverse phase phosphoprotein assay (RPPA).

## Hypothesis

We postulated that inhibition of AR with enzalutamide in combination with the ER degrader fulvestrant, would increase the percentage of patients with limited residual tumor at time of surgery as measured by modified preoperative endocrine predictive index (PEPI) score compared to fulvestrant alone.

## Study Design



Historically, neoadjuvant aromatase inhibitors for 4 months achieved PEPI=0 (defined as tumor size <2 cm, negative nodes, Ki67 <2.7%, ER+) or modified PEPI (calculated without ER consideration due to use of an ER degrader) in ~16% of patients.

Target for this trial was 32% for the combination (fulvestrant plus enzalutamide treatment (Combo) experimental arm.

- Simon 2-stage design for Combination arm:
  - If  $\leq 3$  PEPI = 0 in first 22 patients, then terminate
  - If  $\geq 4$  PEPI = 0, increase arm size to 34 patients
  - Probability early termination 0.52 with 80% power with type I error rate of 0.08

## Results

ER and PR Decreased Significantly by W5 and Time of Surgery in Both Arms, while AR and GR Decreased Only in Combo (Fulv +Enza) Arm at Time of Surgery

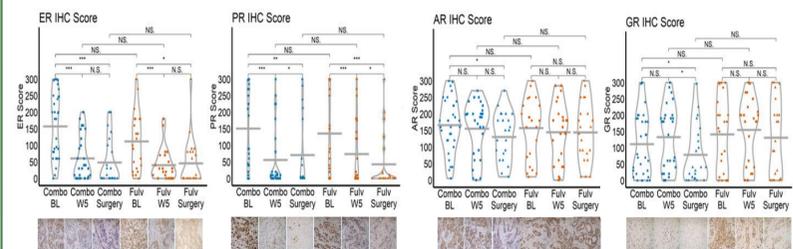


Figure 1. IHC for estrogen, progesterone, androgen and glucocorticoid receptors (ER, PR, AR, GR) by treatment arm and time (baseline, week 5 and time of surgery). ER, PR significantly decreased in both Combo and Fulv only arm, while AR and GR significantly decreased only in the Combo arm by time of surgery. Points represent individual observations and differences in mean indicated by horizontal lines. T-tests compared combo and fulv arms for each outcome and timepoint. Paired t-tests evaluated within-group change over time. "N.S.", "\*\*\*\*", and "\*\*\*\*\*" indicate non-significant, (p>0.05), p<0.05, p<0.01, and p<0.001. Examples are shown for one patient's serial biopsies over time for each protein. Mag =400x

## Funding and Acknowledgements

Supported by DOD BCRP CTR A W81SWH-13-1-0090/91 to JKR/ADE as initiating and partnering PIs. Enzalutamide provided by Astellas and Pfizer. We acknowledge use of the University of Colorado Cancer Center Support Grant P30CA046934 Shared Resources, particularly the Pathology Shared Resource (RRID: SCR\_021994), Biorepository (RRID: SCR\_021989), Biostatistics (RRID: SCR\_021981) and Bioinformatics (RRID: SCR\_021983), Mass Spectrometry Metabolomics Shared Resource (RRID: SCR\_021988), as well as the Human Immune Monitoring Shared Resource (RRID:SCR\_021985). We also wish to thank patient advocates Jane Perlmutter, PhD, MBA, Karen Raines Hunt, PhD, and honor the memory of third patient advocate, Vicki Tosher for valuable input. We are grateful to all who enrolled in NCT02955394 for participating.

## Patient Characteristics (Left) and Univariate Associations with Response (Right)

	Combo Arm (N=33)	Fulv Arm (N=26)	Total (N=59)	PEPI=0 (N=8)
Age at Consent (Years)				
Median (Range)	63 (41, 78)	61 (32, 83)	63 (22, 83)	66 (49, 76)
ECOG PS				
Median (Range)	0 (0, 1)	0 (0, 2)	0 (0, 2)	0 (0, 1)
T Stage				
T2	26 (78.8%)	21 (80.8%)	47 (79.7%)	9 (100.0%)
T3	6 (18.2%)	5 (19.2%)	11 (18.6%)	1 (12.5%)
T4	1 (3.0%)	0 (0.0%)	1 (1.7%)	0 (0.0%)
N Stage				
NX	1 (3.0%)	0 (0.0%)	1 (1.7%)	0 (0.0%)
N0	14 (42.4%)	13 (50%)	27 (45.8%)	10 (100.0%)
N1	17 (51.5%)	13 (50%)	30 (50.8%)	0 (0.0%)
N2	1 (3.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
ER/PR expression				
Positive*	3 (9.1%)	1 (3.8%)	4 (6.8%)	2 (25.0%)
ER+ & PR+	30 (90.9%)	25 (96.2%)	55 (93.2%)	8 (100.0%)
N	27	22	49	9
Median (Range)	80 (10, 100)	85 (10, 100)	80 (10, 100)	90 (10, 100)
Ki67 (%)				
N	29	21	50	10
Median (Range)	12 (1, 80)	10 (1, 60)	12 (1, 80)	5 (1, 15)
History				
IDC	25 (75.8%)	20 (76.9%)	45 (76.3%)	5 (50.0%)
ILC	5 (15.2%)	6 (23.1%)	11 (18.6%)	4 (40.0%)
Other	3 (9.1%)	0 (0.0%)	3 (5.1%)	1 (10.0%)

\*"Yes" indicates patients who had surgery during the study and "No" indicates patients who did not have surgery during the study. "Three patients did not have surgery: one was deceased, and two received additional therapy prior to surgery due to laboratory decisions."  
 \*\*"100%" indicates patients who had low Ki67 (<10%) as measured by IDC at each time point. High Ki67 is defined as >10%.  
 \*The High BL, Low W5 group are designated as responders by Ki67.  
 \*% is provided for variables with less than 20 observations, and the percentage shown is out of the non-missing total. N is presented for variables with less than 20 observations.

## RPPA Revealed Proteins Differentially Altered (BL versus W5) in Responsive (High BL Ki67 >10%, Low W5 <=10%) vs Non-Responsive (High BL/High W5) Tumors

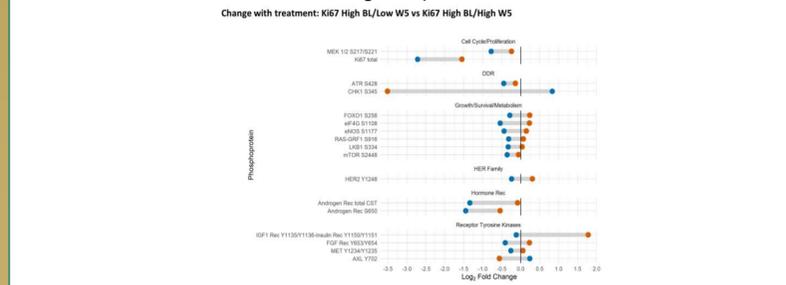


Figure 2. RPPA from frozen laser-captured tumor biopsies (BL vs W5) showed that AR decreased in Responsive Tumors, while Non-Responsive Tumors activated IGF1 Receptor/Insulin Receptor increased as did growth/survival and metabolism proteins. Blue are High BL/LowW5 responsive tumors (N=13) and red High BL/High W5 non-responsive tumors (N=9). Presented proteins were significantly (p<0.1) differentially altered by Empirical Bayes moderated t-test. Dumbbell plots show mean log2 fold change with treatment (W5 - BL) for each protein.

## Proteins Differentially Altered by Treatment in Responsive (PEPI=0) Tumors versus Non-Responsive (PEPI>0) Tumors

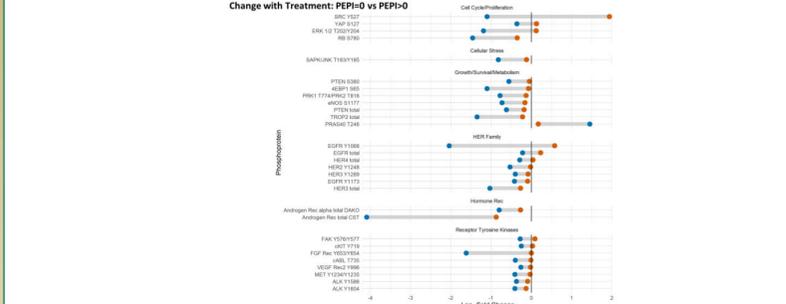


Figure 3. Responsive PEPI=0 (blue, N=9) versus non-responsive PEPI>0 (red, N=45) tumors showed AR, pSRC, pEGFR, pFGFR, and pHER2 to be decreased significantly more by W5 in responsive tumors. Proteins significantly (p<0.1) differentially expressed by Empirical Bayes moderated t-test. Dumbbell plot of the mean log2 change with treatment (W5 - BL) among patients with PEPI=0 and PEPI>0 for each protein.

## Gene Expression Analyses Showed Strong Activation of the Immune System Only in the Combo Treatment Arm

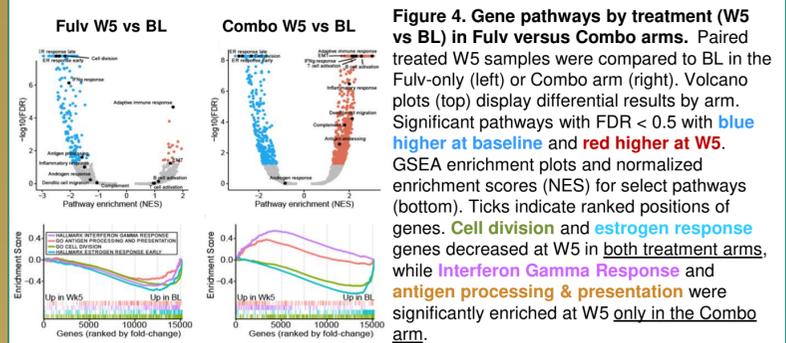


Figure 4. Gene pathways by treatment (W5 vs BL) in Fulv versus Combo arms. Paired treated W5 samples were compared to BL in the Fulv-only (left) or Combo arm (right). Volcano plots (top) display differential results by arm. Significant pathways with FDR < 0.5 with blue higher at baseline and red higher at W5. GSEA enrichment plots and normalized enrichment scores (NES) for select pathways (bottom). Ticks indicate ranked positions of genes. Cell division and estrogen response genes decreased at W5 in both treatment arms, while Interferon Gamma Response and antigen processing & presentation were significantly enriched at W5 only in the Combo arm.

## T Regs, Tumor Associated Macrophages, and MDSCs Decreased Significantly (p<0.05) between BL and W5 and/or by Time of Surgery Only in the Combo Arm

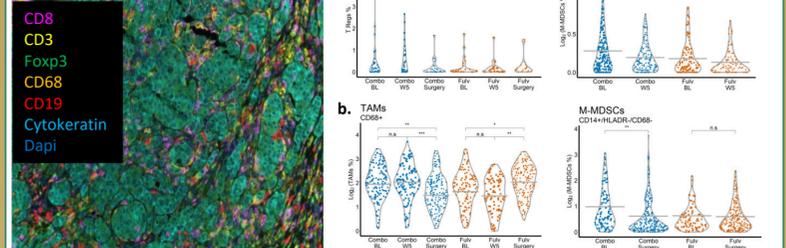


Figure 5. Multiplex IHC showed that T regs, tumor associated macrophages (TAM) and myeloid derived suppressor cells (MDSCs) decreased significantly between BL and W5 and/or time of surgery uniquely in the Combo arm. Results from Akoya Opal technology IHC shown as violin plots stratified by treatment arm (Combo, Fulv) and time (BL, W5, Surgery). a. T Regulatory cells (CD3+CD8-Foxp3+) compared over time by Wilcoxon signed rank tests with points showing patient-level averages (N=53). b. TAMs (CD68+) (N=53) c. For MDSCs (CD14+HLADR-CD68) BL was compared to W5 (top) (N=59, 576 images) and to surgery (bottom) (N=45) separately using two different panels. For b and c, group averages (horizontal lines) and Z-test p-values were calculated from linear mixed effect models (LMM). For all plots p>0.05, p<0.05, p<0.01, and p<0.001 indicated by 'n.s.', '\*', '\*\*', and '\*\*\*\*', respectively.

## Surgical Resections Showed Significantly more Tertiary Lymphoid Structures (TLS) in Combo vs Fulv Only Arm and in Tumors that Achieved PEPI=0

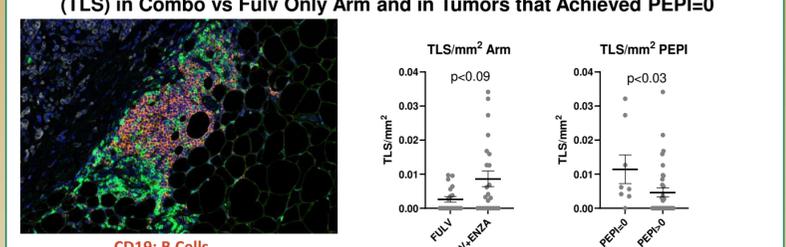


Figure 6. Exploratory quantification of Tertiary Lymphoid Structures (TLS) in surgical resection sections. Multiplex IHC for CD3, CD8, Foxp3, CD68, CD19, and cytokeratin was performed from Fulv only versus Combination arm using Akoya Opal technology. Tertiary Lymphoid structures defined as areas of CD19+ cells (orange) surrounded by CD3+ cells (green) calculated as TLS count per section divided by the total tissue area examined in mm<sup>2</sup>. Mann-Whitney Test was performed to compare groups.

## Univariate Predictor Analysis Showed that the Odds of Response were 4.6-Fold Higher for ILC Versus IDC Tumors

Univariate Predictors	N	OR†	95% CI	p-value
Age at Consent (Years)	59	1.05	0.99, 1.13	0.13
Lab AR (%)				
Baseline Lab AR (%)	55	1	0.98, 1.03	0.96
Week 5 Lab AR (%)	51	1	0.97, 1.02	0.71
Surgery Lab AR (%)	50	0.99	0.97, 1.01	0.34
Lab ER (%)				
Baseline Lab ER (%)	55	0.99	0.97, 1.01	0.47
Week 5 Lab ER (%)	51	0.99	0.96, 1.01	0.3
Surgery Lab ER (%)	50	0.99	0.97, 1.01	0.34
Ki67 (%)				
Baseline Ki67 (%)	50	0.9	0.79, 0.98	0.004
Week 5 Ki67 (%)	49	0.62	0.29, 0.95	0.005
W5-BL Change in Lab Ki67 (%)	44	1.05	0.96, 1.16	0.24
History				
Invasive Ductal Carcinoma (IDC)	45			
Invasive Lobular Carcinoma (ILC)	11	4.57	0.94, 21.97	0.059

OR = Odds Ratio, CI = Confidence Interval  
 †Univariate logistic regression models with PEPI score (PEPI=0 vs reference: PEPI>1) as the outcome are presented with points (blue for ILC and orange for IDC) and likelihood ratios (95% CI) in parentheses. Odds ratios above one indicate increases in the odds of response (PEPI=0) and odds ratios below one indicate decreases in the odds of response.  
 \*While Lab ER at the time of surgery was included in the PEPI score, other components of PEPI were distinct whether patients went 0 or non-zero.

- Key univariate take-aways:
- Each % point higher W5 Ki67 was associated with lower odds of PEPI=0
  - The odds of PEPI=0 were 4.6-fold higher for ILC vs IDC tumors

## Proteins Differentially Decreased by Treatment in ILC vs IDC included AR, Survival/Metabolism/Cell Cycle Proteins, Activated Growth Factor Receptors (pHER family, pRET, pFAK, pFGFR) and ribosomal S6 kinase (p70S6K)

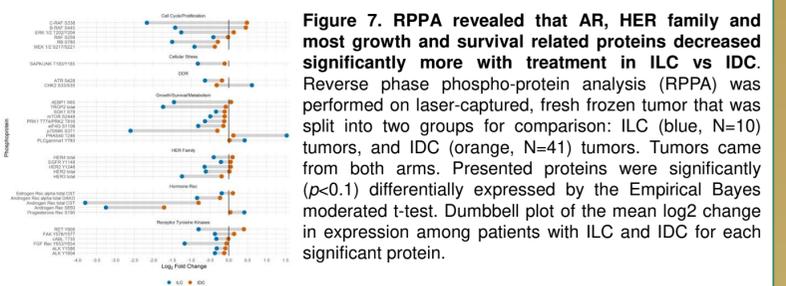


Figure 7. RPPA revealed that AR, HER family and most growth and survival related proteins decreased significantly more with treatment in ILC vs IDC. Reverse phase phospho-protein analysis (RPPA) was performed on laser-captured, fresh frozen tumor that was split into two groups for comparison: ILC (blue, N=10) tumors, and IDC (orange, N=41) tumors. Tumors came from both arms. Presented proteins were significantly (p<0.1) differentially expressed by the Empirical Bayes moderated t-test. Dumbbell plot of the mean log2 change in expression among patients with ILC and IDC for each significant protein.

## MDSCs Decrease with Treatment in both ILC and IDC (BL vs W5)

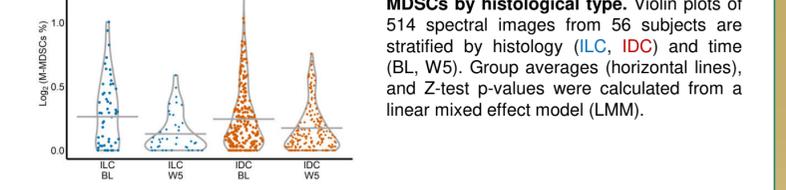


Figure 8. Multiplex IHC results for M-MDSCs by histological type. Violin plots of 514 spectral images from 56 subjects are stratified by histology (ILC, IDC) and time (BL, W5). Group averages (horizontal lines), and Z-test p-values were calculated from a linear mixed effect model (LMM).

## Conclusions

- No new toxicities, most grade 1. Mild reversible cognitive impairment and occasional tremor associated with enzalutamide (Elias AD *et al* *NPJ Breast Cancer* 2024 Oct PMID: 39368973).
- PEPI=0 was achieved more frequently on the Combo arm (8/33) than Fulv only arm (2/26), although this difference (p=0.16) did not meet the pre-specified statistical significance (p=0.08).
- ER, PR, and Ki67 levels decreased by W5 and time of surgery. AR and GR significantly decreased by time of surgery only in the Combination (fulvestrant plus enzalutamide) arm.
- None of the 51% LN+ converted to PEPI=0 (similar to other pre-op endocrine protocols).
- Total ar, pSRC, pEGFR, pFGFR, pHER2 and pHER2 are among the proteins most differentially decreased by treatment in PEPI=0 tumors.
- Combining an anti-androgen, enzalutamide, with fulvestrant induced an active immune gene signature, increased # of TLS and decreased immune-suppressive T Regs, TAMs, and MDSCs.
- Anti-androgens may be particularly useful for patients with ILC, although future studies with larger patient numbers would need to be conducted to confirm these results.