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TITLE: Targeting Androgen Receptor in Breast Cancer: Enzalutamide as a Novel Breast Cancer Therapeutic

PRINCIPAL INVESTIGATOR: Dr. Anthony Elias

CONTRACTING ORGANIZATION: University of Colorado Denver

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14. ABSTRACT

Enzalutamide has clinical activity in breast cancer as a single agent and in combination with exemestane. Activity is seen in both triple negative AR+ BC and also ER+AR+ BC. Clinical data in Her2+ AR+ BC is too immature to make conclusions. The proposed clinical trials for Years 3-8 are justified based on clinical activity and the current preclinical data.

15. SUBJECT TERMS

Breast cancer (BC) subtypes; androgen receptor (AR); preclinical modeling; enzalutamide; AR inhibition; resistance mechanisms; predictive biomarkers.

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Award Number W81XWH-13-1-0091 Final Report

Title: Targeting Androgen Receptor in Breast Cancer: Enzalutamide as a Novel Breast Cancer Therapeutic

Collaborating/Partnering PI: Anthony D Elias, MD

Contracting Organization: University of Colorado Anschutz Medical Campus

Report Date: 8/15/2013-2/14/2023

Type of Report: Final Progress Report

Introduction

The central thesis of this grant is to understand the role of AR signaling in breast cancer subtypes, and understand how to best use an inhibitor of AR signaling, enzalutamide (enza), as a therapeutic agent in breast cancer. With the recognition that AR is expressed in all subtypes of breast cancer, that overexpression is frequently associated with relative resistance to therapy (both anti-estrogen and chemotherapy) (work of our group and others), and with the advent of increasingly potent AR signaling inhibitors in prostate cancer, the area of anti-AR therapeutics in breast cancer is one of the most active worldwide. The preclinical portion of this grant serves to understand mechanism of action of AR signaling inhibition alone or in combination with other targeted agents in ER+, Her2+, or TNBC in preclinical models, and then perform biomarker analysis in human tissues obtained before, during and after treatment with enzalutamide. The clinical portion of this grant serves to obtain these tissues in concert with the overall clinical development of enzalutamide in the subtypes of breast cancer.

Keywords

Breast cancer (BC) subtypes; androgen receptor (AR); preclinical modeling; enzalutamide; AR inhibition; resistance mechanisms; predictive biomarkers; targeted therapy.

Overall Project Summary

Clinical Aim 1: To identify pretreatment molecular characteristics associated with lack of response and/or prolonged PFS (Patient Tissues).
Clinical Aim 2: To determine if a decrease in Ki67 or increase in apoptosis as measured by TUNEL in biopsies taken before treatment as compared to after 2-4 weeks of treatment or other to be determined genes or proteins are associated with lack of response and/or prolonged PFS.
Clinical Aim 3: To determine if changes in molecular determinants between pre-treatment biopsies and tissue at time of disease progression can help identify resistance mechanisms.
Task 1: Serial Biopsy Trial (Elias, Traina, Schwartzberg, Petricoin, Patient advocates, Richer)

- The DOD sponsored serial biopsy trial titled "Exploratory Development of Predictive Biomarkers for Patients with Androgen-Receptor Positive (AR) Breast Cancer (BC) Treated with Enzalutamide (MDV3100); COMIRB 13-1473," was activated at the University of Colorado site and the West Clinic/University of Tennessee site. It is now closed to accrual at all sites due to the reasons stated below.
- 6 patients with serial biopsies were enrolled.
    - Tissues studies were reported in previous years' Progress Report.

    - Please see the Annual Report for Award Number W81XWH-13-1-0090 for all laboratory work done on the tissues and for all preclinical work.
- Accrual to this trial was not completed due to the limitation that this biopsy trial was a companion to therapeutic trials of enzalutamide in breast cancer sponsored by Medivation and Astellas. Because those therapeutic trials were opened to large numbers of institutions that were not part of our DOD grant, and were completed very quickly, our accrual was limited.
- On the other hand, the clinical development of enzalutamide was enhanced, making possible the forward-thinking investigator-sponsored trials as outlined for Years 3-5 in our grant.
- The trials in Clinical Aims 4 and 5 did include serial biopsies, and allowed us to complete the Clinical Aims 1-3 albeit not with patients treated with single agent enzalutamide.
- Please see the Annual Report for Award Number W81XWH-13-1-0090 for all laboratory work done on the tissues and for all preclinical work.
- Heterogeneous patients with respect to ER status, prior treatment.
- ER/AR IHC from archived tissue (obtained from primary tumor) often dramatically different from the immediate pre-treatment biopsy.
- Two patients with ER+ disease had mutated ESR1 in the pretreatment biopsy
- Using RPPA methodology to examine phosphoproteins, pre- vs post-enzalutamide tissues frequently demonstrated downregulation of various growth factor pathways.

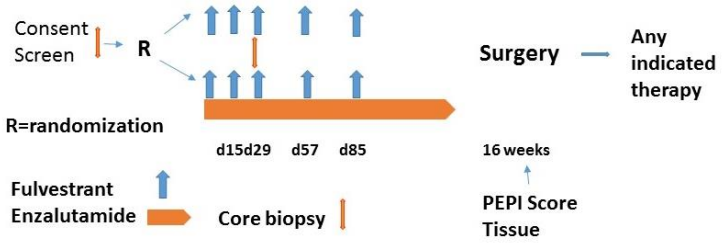
Clinical Aim 4: To determine if enza can overcome *de novo* resistance to exemestane in postmenopausal women with T2 or larger ER+ BC treated preoperatively.

Task 1: Trial II: Randomized Preoperative trial in AR+/ER+ BC (Elias, LoRusso, Traina, advocates, Richer)

- Written protocol completed Month 21
- Submitted to Scientific Review Committee Month 21
- Submitted to IRBs (all institutions) Month 22
- DoD Human Research Protection Office (HRPO) Month 24

The Clinical Aim 4 trial was originally a randomized phase II trial of exemestane +/- enzalutamide given preoperatively for women with T2 or larger AR+/ER+ BC. This LOI was submitted to Astellas/Medivation in August 2015. Unfortunately, before it was evaluated, Astellas shut down their IIT program pending an internal assessment of their development plans for enzalutamide in breast cancer. This LOI was ultimately evaluated and rejected in 12/2015. An LOI for the same trial using fulvestrant +/- enzalutamide was submitted in 1/2016 and accepted by Astellas in 2/2016. The full protocol was written and submitted to Astellas and to the UCCC Scientific Review Committee in May 2016. Because >90% of ER+ BC is also AR+, AR IHC is not being used to select patients.

Preoperative Fulvestrant +/- Enzalutamide
Eligibility: >=T2; ER+/Her2-



If pre- or perimenopausal, goserelin 3.6 mg sq every 4 weeks (or equivalent)
Samples to be collected:

- Tumor tissue: at baseline, at 4 weeks, at time of surgery (16 weeks)
- Plasma: at baseline, at 4 weeks, at time of surgery (16 weeks)

- Found to be IND exempt by FDA in 8/2016
- COMIRB approved 9/2016 – Protocol 16-1042
- HRPO approved 4/2017
- Astellas approved 10/2016
- Astellas Drug Supply contract and IP agreement approved 8/2017
- Trial activated at University of Colorado 8/30/17
- Patient #1 accrued 10/2/17
- Trial open to accrual at MSKCC 7/2/18
- Trial open to accrual at University of Tennessee 9/17/18
- **69 patients consented. 62 enrolled (3 screen failures and 4 withdrawals prior to enrollment), and 61 randomized and treated (1 patient withdrew prior to treatment). Stage I accrual was completed 3/2/20, with 22 evaluable subjects enrolled on the experimental (combination) arm. More than 4 patients on the combination arm have achieved a PEPI score = 0, therefore an additional 16 patients were enrolled in stage 2. Stage 2 accrual was completed on 10/14/2021. 5 year follow-up for relapse and survival is continuing as per approved protocol.**
- **SAEs that were possibly related: G4 MI (1)**
- **SAEs felt to be unrelated: G3 gallbladder obstruction (1); G3 hematoma from breast surgery**

Primary endpoint: To evaluate whether the addition of enzalutamide to fulvestrant treatment for ~4 months in women with ≥T2 ER+/Her2- BC will achieve a PEPI score of 0 at time of surgery in 32%. PEPI (preoperative endocrine prognostic index) is a model that combines ER, pathologic tumor site, nodal status, and Ki67 score at time of surgery to predict subsequent risk of recurrence. PEPI = 0 means ypT < 1cm; Ki67 <10%; N0; ER+. PEPI is used to guide postoperative therapy in a number of neoadjuvant breast cancer trials (NCT01723774, NCT02236572, NCT01923168, NCT01953588). Fulvestrant has been shown to be more active against ER+ MBC than AIs in the randomized phase II FIRST trial. Phase III confirmation of fulvestrant vs. AI in MBC in the FALCON trial (NCT01602380). The ALTERNATE trial, accruing in NCTN, randomizes women with cT2-4 N0-3 M0 ER+/Her- breast cancer to anastrozole, fulvestrant, or the combination for 6 months prior to surgery (NCT01953588).

Statistical design: From the literature, preoperative aromatase inhibitor (AI) achieved PEPI score of 0 in 16%. We expect to achieve PEPI score of 0 in 32% for fulvestrant plus enzalutamide. Fulvestrant alone would likely be intermediate. We are using a Simon 2-stage design for the combination (experimental) arm: if ≤ 3 PEPI = 0 in first 22 evaluable patients, then will terminate entire trial. If ≥ 4 achieve PEPI = 0, then will increase arm size to 34. The probability of early termination is 0.52. We will have an 80% power with a type I error rate of 0.08. The trial has a concurrent control arm of fulvestrant alone with 27 patients. If the true PEPI = 0 is >16%, the observable rate has a 90% likelihood of being > 6%. We anticipate ~10% inadequate tissue specimens. A total of 49-61 patients will be randomized and treated.

Grant Hypotheses (from serial tissue biopsies):

- Decrease in Ki67 after ~4 weeks of treatment to below 10% will be associated with response to therapy and will correlate with improved PEPI scores (Clinical Aim 2).

- Certain pretreatment molecular characteristics (such as AR:ER ratio in ER+ tumors, Her2 status, PI3K pathway mutations, or others) will be associated with lesser response and poorer PEPI score (Clinical Aim 1).
- High AR expression will be associated with resistance to anti-estrogen therapy. Its blockade may enhance response (Clinical Aim 3).

Eligibility: At least 18 years of age, ER+/Her2- BC (>90% will be AR+), stage ≥cT2, planned to get local surgery, PS 0-2, safe to biopsy, no prior treatment. Women must be postmenopausal, or if pre- or peri-menopausal, will require concurrent ovarian suppression.

Clinical Results: Overall 69 patients were consented, of whom 59 were evaluable (33 on combination arm and 26 on the fulvestrant only arm). Upon completion of the Simon stage I portion of the experimental arm, 4/22 patients had achieved PEPI = 0, whereupon the second stage completed accrual. Of the 33 patients treated on the FE arm, median age was 63. At baseline, 21 had T3/T4 tumors, 91% were ER+/PR+, median AR expression was 80%, median Ki67 was 15%. Of the 26 patients treated on the F arm, median age was 61, 19% had T3/T4 tumors, 96% were ER+/PR+, median AR expression was 85%, median Ki67 was 10%. Toxicity was as expected with endocrine therapy. Surgery was completed in 95% and PEPI = 0 achieved in 10/59 (17%). PEPI=0 was achieved more frequently on the FE arm (8/33, 24%) than the F arm (2/26, 8%) (p = 0.16).

IHC comparison of baseline and Wk5 biopsies showed decreased ER, PR, and Ki67 levels by Wk5 that remained decreased at time of surgery. AR and GR were significantly decreased only at the time of surgery and only in the combination arm. RPPA revealed that the average baseline EGFR (Y1604 and Y992) was higher among pts with PEPI=0 tumors, whereas the average baseline mTOR activation was higher among pts with PEPI>0. Significant changes in protein levels with treatment and between tumors that went from a high to low ≤ 10% Ki67 and between the PEPI=0 versus PEPI>0 included AR as the most significantly decreased by treatment in tumors with PEPI=0 as compared to PEPI>0 tumors. Polaris multiplex immunofluorescence analyses demonstrated that myeloid-derived suppressor cells (MDSC) were significantly reduced by treatment only in the FE arm.

Conclusions: The combination of FE had manageable side effects. PEPI=0 was achieved more frequently on the FE arm (8/33) than the F arm (2/26), although this difference (p=0.16) did not meet the pre-specified statistical significance. It is notable that we did not observe evidence for tumor stimulation with AR blockade.

EGFR pathway activation was higher in pretreatment tumor for those pts achieving PEPI=0; however, mTOR pathway activation was higher in pretreatment tumor for those pts with poor histologic response, an observation also observed in our trial of FE in pts with ER+/HER2- metastatic disease (SABCS 2021). When comparing pretreatment tumor to tumor after 4 weeks therapy, total AR by RPPA was the most differentially decreased protein in those pts who achieved PEPI=0 status. AR signaling is known to support immunosuppressive cells in the microenvironment, and we observed a marked decrease in MDSCs with treatment only on the FE arm (AACR 2023 poster and manuscript in preparation).

Clinical Aim 5: To determine the maximum tolerated dose and toxicity of enza when combined with the most promising combinations as defined in the preclinical modeling experiments during Years 1-2. As an example, a combination of enza with everolimus +/- a chemotherapy agent in previously treated metastatic TNBC.

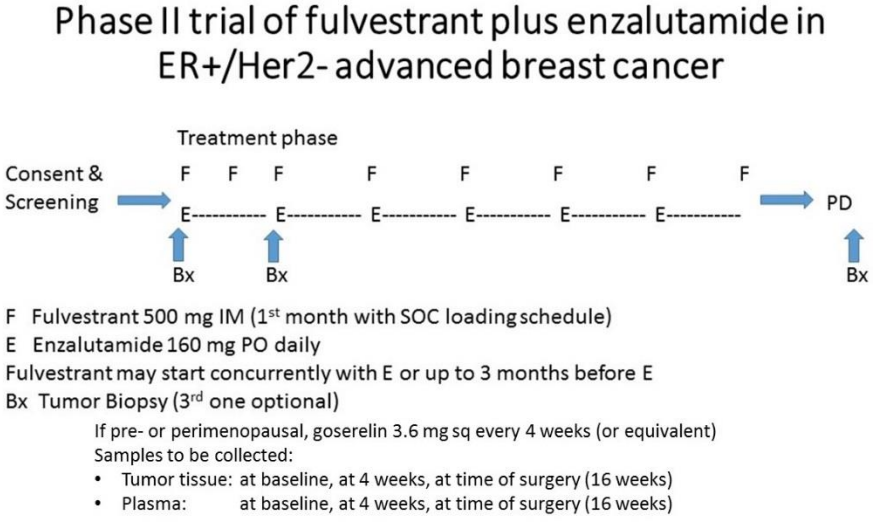
Task 1: Trial III: Phase II trial in ER+ metastatic BC: Enzalutamide plus everolimus (Traina, Elias, LoRusso, advocates, Richer)

- Written protocol completed Month 21
- Submitted to Scientific Review Committee Month 21
- Submitted to IRBs (all institutions) Month 22

- DoD Human Research Protection Office (HRPO) Month 24

Support for the original trial of enzalutamide plus everolimus for AR+ TNBC was not granted by Medivation and Astellas. An alternative trial, as presented at the Milestone Meeting in May 2015, was a Phase I/II trial in ER+ BC: Enzalutamide plus exemestane or fulvestrant with the addition of either everolimus or palbociclib. This LOI was submitted to Medivation and Astellas and was rejected for support or drug supply. Ultimately, once the phase I of fulvestrant plus enzalutamide was completed and reported, the following phase II trial was approved, then submitted for an FDA IND application in 8/2016. It was declared IND exempt by CTEP.

- IRB (COMIRB 16-1001) approved 10/2016
- HRPO approved 4/2017
- Submitted to regulatory committee at University of Tennessee 11/2016
- Astellas approved 10/2016
- Astellas Drug Supply contract and IP agreement approved 6/2017
- Trial activated at University of Colorado 6/29/17
- Trial activated at University of Tennessee 9/17/18
- Monthly teleconferences to review patients, safety, and research progress. Also have a trial monitor (Dr. Lam).
- **Accrual completed November 2019 after reaching goal of 20 evaluable subjects**
- **SAEs felt to be possibly related: None**
- **SAEs felt to be unrelated: G2 pleural effusion (2), G3 seizure (1), G3 fracture (1); G3 pain (2); G2 gallbladder obstruction (1); G3 increased AST (1); G4 hypercalcemia with hypokalemia, anemia and fatigue (1); G3 overdose; G3 dehydration and UTI (1); G3 biliary obstruction (1); G2 basal cell carcinoma right lower eyelid (1); G3 vomiting with G4 hypotension**



Primary Endpoints: To determine the clinical benefit rate (CBR) of adding enzalutamide to fulvestrant treatment in women with ER+/Her2- advanced BC. To evaluate the safety and tolerability of fulvestrant plus enzalutamide.

Statistical Design: Open label single arm phase II trial with 24 patients. Undesirable clinical benefit rate (CBR at 24 weeks) would be ≤10%. Desirable CBR would be ≥30%. This would provide 89% power with a one-sided α of 0.085. If ≥5/24 patients have CBR of ≥24 weeks, then combination warrants further evaluation. We anticipate 10% inadequate tissue specimens.

Grant Hypotheses (from serial tissue biopsies):

- Decrease in Ki67 after ~4 weeks of treatment to below 10% will be associated with response to therapy and will correlate with CBR (Clinical Aim 2).
- Pretreatment molecular characteristics (such as AR:ER ratio in ER+ tumors, Her2 status, PI3K pathway mutations, or others) will be associated with CBR (Clinical Aim 1).

- High AR expression will be associated with resistance to anti-estrogen therapy. Its blockade may enhance response (Clinical Aim 3).
- Tissue at time of disease progression will be enriched for genes/proteins/mutations representing resistance mechanisms

Eligibility: At least 18 years of age, ER+/Her2- BC (>90% will be AR+), metastatic, tumor tissue available and safe for serial biopsy, candidate for fulvestrant therapy, PS 0-2, safe to biopsy, no CNS disease. Women must be postmenopausal, or if pre- or peri-menopausal, will require concurrent ovarian suppression.

Clinical Results: Of the 32 eligible participants, 28 were evaluable for response. The median age was 61 [46-87] years and the median ECOG PS was 1 [0-1]. Patients were heavily pretreated with a median of 3 [1,9] prior hormonal agents and 4 [0,8] prior non-hormonal therapies. Twelve (37.5%) patients had prior fulvestrant and 29 (90.6%) had visceral disease. Treatment emergent adverse events were consistent with what would be expected with hormonal therapy. At week 24, 7 (25.0%) (95% CI: 10.7 to 44.9) participants had stable disease and no participants had partial response**.** The median time to progression was 8 weeks (95% CI: 8 to 20).

Significant ($p<0.2$) univariate relationships were observed between continuous PFS and the following variables: ER and AR percent positivity by IHC, and PIK3CA and/or PTEN mutations. Specifically, the average hazard for progression was 4.32 (95% CI: 1.46 to 12.83) times higher for participants with ER < 10 percent positive compared to participants with ER $\geq$ 10 percent positive ($p=0.008$). This association remained significant when ER was considered continuous ($p=0.024$). Additionally, the average hazard of progression was 2.28 (95% CI: 0.93 to 5.62) times higher for participants with AR < 10 percent positive compared to participants with AR $\geq$ 10 percent positive ($p=0.073$). The hazard of disease progression for participants with AR and/or ER <10% positive was 2.35 (95% CI: 0.99 to 5.58; $p=0.052$) times the hazard for participants with AR and/or ER ≥ 10% positive.

When comparing phosphorylated proteins in the in the "Short PFS" defined in all RPPA analyses as PFS ≤ 60 days (n=20) to those who experienced "Long PFS" defined as PFS>24 weeks (168 days) (n=7), the mTOR pathway had higher baseline expression levels the tumors of those with Short PFS. According to robust moderated t-tests, baseline mTOR S2448 ($p=0.008$), eNOS/NOSIII S116 ($p=0.029$), S6RP S240/S244 ($p=0.031$), eIF4G S118 ($p=0.037$), and p7056K T389 ($p=0.043$) were significantly higher in the Short PFS group compared to the Long PFS group **(Figure 2A&B).** Similar results were obtained when Short PFS was defined as <24 weeks (n=25). This suggests mTOR activation in the pre-treatment tumor biopsies from patients with a short time to progression.

Future trials could be done in second line endocrine therapy (progressing after AI and cdk4/6 inhibition) and in PI3K wild type tumors to evaluate fulvestrant plus everolimus with or without an AR signaling blocker.

Key Research Accomplishments:

Enzalutamide has clinical activity in breast cancer as a single agent and in combination with endocrine therapies.

Two clinical trials were submitted to our local IRB (COMIRB 16-1042 and COMIRB 16-1001) to complete the clinical aims of this grant. Both were activated for clinical accrual at University of Colorado. 38 patients signed consent for COMIRB 16-1001 (Clinical Aim 5), of whom 6 screen failed. The 16-1001 trial completed accrual in

November 2019. 69 patients signed consent for COMIRB 16-1042 (Clinical Aim 4) of whom 3 screen failed and 5 withdrew prior to treatment. The 16-1042 trial completed accrual in October 2021 with last patient off protocol mandated treatment by March 2022.

Please see the Annual Report for Award Number W81XWH-13-1-0090 for all laboratory work done on the tissues and for all preclinical work.

Conclusion:

Enzalutamide has clinical activity in breast cancer as a single agent and in combination with exemestane. Activity is seen in both triple negative AR+ BC and also ER+AR+ BC. Clinical data in Her2+ AR+ BC is too immature to make conclusions. The proposed clinical trials for Years 3-9 were justified based on clinical activity and the current preclinical data.

Publications, Abstracts, and Presentations:

Papers:
Dawn R. Cochrane, Sebastian Bernales, Britta M. Jacobsen, Diana M. Cittelly, Erin N. Howe, Nicholas C. D'Amato, Nicole S. Spoelstra, Annie Jean, Paul Jedlicka, Kathleen C. Torkko, Andy Protter, Anthony D. Elias and J. K. Richer. Role of the Androgen Receptor in Breast Cancer and Preclinical Analysis of Enzalutamide. BREAST CANCER RESEARCH 2014 Jan 22;16(1). PMID: 24451109

Designated as Highly Cited by the journal Breast Cancer Research.

Barton VN, D'Amato NC, Gordon MA, Lind HT, Spoelstra NS, Babbs B, Heinz RE, Elias AD, Jedlicka P, Jacobsen BM, Richer JK. Multiple molecular subtypes of triple negative breast cancer depend on androgen receptor for proliferation and invasion. Molecular Cancer Therapeutics 2015; 14: 769-778. PMID: 25713333

Barton VN, Gordon MA, Christenson JL, D'Amato NC, Elias A, Richer JK. Androgen receptor biology in triple negative breast cancer: a case for AR+ and quadruple negative disease subtypes. Horm Cancer 2015 Jul 23, epub ahead of print. PMID: 26201402.

D'Amato NC, Gordon MA, Babbs B, Spoelstra NS, Carson Butterfield KT, Torkko KC, Phan VT, Barton VN, Rogers TJ, Sartorius CA, Elias A, Gertz J, Jacobsen BM, Richer JK. Cooperative Dynamics of AR and ER Activity in Breast Cancer. Mol Cancer Res. 2016; 14: 1054-1067. Aug 26. pii: molcanres.0167.2016. [Epub ahead of print] PMID: 27565181 (Figure on cover).

Christenson JL, Trepel JB, Eli HY, Lee S, Eisner JR, Baskin-Bey ES, Elias AD, Richer JK. Harnessing a different dependency: how to identify and target androgen receptor-positive versus quadruple-negative breast cancer. Horm cancer 2018 Jan 16. doi: 10.1007/s12672-017-0314-5. [Epub ahead of print] Review. PMID: 29340907

Williams MM, Spoelstra NS, Arnesen S, O'Neill KI, Christenson JL, Reese J, Torkko KC, Goodspeed A, Rosas E, Hanamura T, Sams SB, Li Z, Oesterreich S, Riggins RB, Jacobsen BM, Elias A, Gertz J, Richer JK. Steroid hormone receptor and infiltrating immune cell status reveals therapeutic vulnerabilities of ESR-1-mutant breast cancer. Cancer Res 2021; Feb 1;81(3):732-746. doi: 10.1158/0008-5472.CAN-20-1200. Epub 2020 Nov 12. PMID: 33184106 PMCID: PMC7854521

Elias AD, Spoelstra NS, Staley AW, Sams S, Crump LS, Vidal GA, Borges VF, Kabos P, Diamond JR, Shagisultanova E, Afghahi A, Mayordomo J, McSpadden T, Crawford G, D'Alessandro A, Zolman KL, van Bokhoven A, Zhuang Y, Gallagher RI, Wulfkuhle JD, Petricoin EF III, Gao D, Richer JK. Phase II trial of fulvestrant plus enzalutamide in ER+/HER2- advanced breast cancer. Npj Breast Cancer 2023, in press.

Abstracts:
Elias AD, Burris HA, Patel MR, Schwartzberg LS, **Richer JK**, Kavalerchik E, Stopatschinskaja S, Gibbons J, Markova D, Steinberg JL, Traina TA. MDV3100-08: a phase I study evaluating the safety and pharmacokinetics of enzalutamide plus fulvestrant in women with advanced hormone receptor-positive breast cancer. Proc SABCS 2015, accepted poster presentation.

Elias AD, Fornier M, Vidal GA, Sams S, Spoelstra N, Kabos P, Diamond JR, Shagisultanova E, Crump LS, Gallagher RI, Wulfkuhle J, Petricoin E, Zolman K, Biller S, Alami V, Staley A, McSpadden T, Borges V, Gao D, Jenifer K. Richer JK. Randomized Phase II Trial of Preoperative Fulvestrant with or without Enzalutamide for ER+/Her2-Breast Cancer. Proc AACR 2023, poster presentation.

Dr. Elias gave the following presentations:
Elias A. What is the androgen receptor doing in breast cancer and can we target it? 14th Annual International Congress on the Future of Breast Cancer. PER. Huntington Beach, CA 7/17/15.
Elias A. The role of AR in breast cancer. 3rd Annual West Cancer Center Oncology Conference. Memphis, TN, 10/27/17.

Inventions, Patents and Licenses: Nothing to report

Reportable Outcomes: Metastatic trial 16-1001 manuscript is in press at npjBreast Cancer. Preoperative trial 16-1042 poster presented at AACR 2023, and manuscript under preparation.

Please see the Annual Report for Award Number W81XWH-13-1-0090 for all laboratory work done on the tissues and for all preclinical work.

Other Achievements: Please see publication/abstract/reference list.

Gordon MA, D'Amato NC, Gu H , Babbs B, Wulfkuhle JD , Petricoin EF, Gallagher RI, Dong T, Torkko KC, Liu B , Elias A and JK Richer. Synergy between androgen receptor antagonism and inhibition of mTOR and HER2 in breast cancer. MOLECULAR CANCER THERAPEUTICS. 2017 Jul;16(7):1389-1400. PMID: 28468774

Barton VN, Christenson JL, Rogers TJ, Butterfield K, Babbs B, Spoelstra NS, D'Amato NC, Elias A, and JK Richer. Androgen receptor supports an anchorage independent, cancer stem cell like population in triple negative breast cancer. CANCER RESEARCH. 2017 Jul 1;77(13):3455-3466. PMID: 28512248

Schwartzberg LS, Yardley DA, Elias AD, Patel M, LoRusso P, Burris HA, Gucalp A, Peterson AC, Blaney ME, Steinberg JL, Gibbons JA, Traina TA. A phase 1/1b study of enzalutamide alone and in combination with hormonal therapies in women with advanced breast cancer. Clin Cancer Res 2017 Aug 1;23(15):4046-4054. doi: 10.1158/1078-0432.CCR-16-2339. Epub 2017 Mar 9. PMID: 28280092

Gordon MA, D'Amato NC, Gu H, Babbs B, Petricoin EF, Wulfkuhle J, Torkko K, Elias A, Richer JK. Synergy between androgen receptor antagonism and inhibition of mTOR and HER2 in breast cancer. Molecular Cancer Res 2017; 16: 1389-1400. PMID: 28468774

Barton V, Christenson J, Rogers T, Butterfield C, Kiel T, Babbs B, Spoelstra N, D'Amato N, Elias A, Richer JK. Androgen receptor supports a cancer stem cell-like population in triple-negative breast cancer. Cancer Research 2017; 77: 3455-3466. PMID: 28512248

Barton VN, Gordon MA, Richer JK, Elias A. Anti-androgen therapy in triple negative breast cancer. Ther Adv Med Oncol 2016; 8: 305-308. PMID: 27482289.

Christenson JL, Trepel JB, Eli HY, Lee S, Eisner JR, Baskin-Bey ES, Elias AD, Richer JK. Harnessing a different dependency: how to identify and target androgen receptor-positive versus quadruple-negative breast cancer. Horm cancer 2018 Jan 16. doi: 10.1007/s12672-017-0314-5. [Epub ahead of print] Review. PMID: 29340907

Gucalp A, Danso M, Elias A, Bardia A, Ali H, Potter D, Gabrail N, Haley B, Khong H, Riley E, Ervin L, Eisner J, Baskin-Bey E, Moore W, Traina TA. Phase 2 Stage 1 clinical activity of seviteronel, a selective CYP17-lyase and androgen receptor inhibitor, in women with advanced AR+ triple negative or estrogen receptor positive breast cancer: CLARITY-01. Proc ASCO 2017, A-1102.

Elias A, Gucalp A, Van Poznak DH, Bardia A, Resaul A, Eisner J, Baskin-Bey E, Traina TA. Initial phase (Ph)2 clinical activity of seviteronel, a selective CYP17-lyase and androgen receptor (AR) inhibitor, in men with advanced breast cancer (BC). Proc SABCS 2017, P5-23-04, poster.

Please see the Annual Report for Award Number W81XWH-13-1-0090 for all laboratory work done on the tissues and for all preclinical work.

References:

Papers:
Dawn R. Cochrane, Sebastian Bernales, Britta M. Jacobsen, Diana M. Cittelly, Erin N. Howe, Nicholas C. D'Amato, Nicole S. Spoelstra, Annie Jean, Paul Jedlicka, Kathleen C. Torkko, Andy Protter, Anthony D. Elias and J. K. Richer. Role of the Androgen Receptor in Breast Cancer and Preclinical Analysis of Enzalutamide. BREAST CANCER RESEARCH 2014 Jan 22;16(1). PMID: 24451109

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Barton VN, D'Amato NC, Gordon MA, Lind HT, Spoelstra NS, Babbs B, Heinz RE, Elias AD, Jedlicka P, Jacobsen BM, Richer JK. Multiple molecular subtypes of triple negative breast cancer critically rely on androgen receptor and respond to Enzalutamide in vivo. Molecular Cancer Therapeutics 2015; 14: 769-778. PMID: 25713333

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Gordon MA, D'Amato NC , Gu H , Babbs B, Wulfkuhle JD , Petricoin EF, Gallagher RI, Dong T, Torkko KC, Liu B , Elias A and JK Richer Synergy between androgen receptor antagonism and inhibition of mTOR and HER2 in breast cancer. MOLECULAR CANCER THERAPEUTICS. 2017 Jul;16(7):1389-1400. PMID: 28468774

Barton VN, Christenson JL, Rogers TJ, Butterfield K, Babbs B, Spoelstra NS, D'Amato NC, Elias A, and JK Richer. Androgen receptor supports an anchorage independent, cancer stem cell like population in triple negative breast cancer. CANCER RESEARCH. 2017 Jul 1;77(13):3455-3466. PMID: 28512248

Schwartzberg LS, Yardley DA, Elias AD, Patel M, LoRusso P, Burris HA, Gucalp A, Peterson AC, Blaney ME, Steinberg JL, Gibbons JA, Traina TA. A phase 1/1b study of enzalutamide alone and in combination with hormonal therapies in women with advanced breast cancer. Clin Cancer Res 2017,. 2017 Aug 1;23(15):4046-4054. PMID: 28280092

Williams MM, Spoelstra NS, Arnesen S , Christenson JL, O'Neill, K, Reese JM, Blanchard ZD, Hanamura T, Jacobsen BM, Elias A, Gertz J, and JK Richer. Androgen receptor activity and other pathways critical to estrogen-independent activity of ESR1 Mutant Metastatic Breast Cancer. In preparation 2019

Abstracts:
D'Amato, NC, D Cochrane, N Spoelstra, A Chitrakar, B Babbs, A Protter, AD Elias, and J Richer. (Mar 2014) Inhibiting Androgen Receptor Nuclear Localization Decreases ER Activity and Tumor Growth in ER+ Breast Cancer. University of Colorado Postdoctoral Research Day, Aurora, CO. * won best overall poster award.

Barton VN, D'Amato N, Gordon M, Elias, A, and JK Richer. Targeting androgen receptor decreases proliferation and invasion in preclinical models of triple negative breast cancer. Presented at University of Colorado Cancer Center Annual Retreat "Novel Experimental Models for Cancer Research," September 2014. * Won outstanding poster award.

Elias A, Richer JK, LoRusso P, Peterson AC, Steinberg J, Mordenti J, Lopez C, Hudis C, Traina T. MDV3100-08: A phase 1 open-label, dose-escalation study evaluating the safety, tolerability, and pharmacokinetics of MDV3100 in women with incurable breast cancer. ASCO 2012, TPS668.

Traina TA, Yardley, DA, Patel M, Schwartzberg L, Elias A, Gucalp A, Peterson AC, Hannah A, Gibbons J, Khondker Z, Hudis CA, LoRusso P. A phase 1 open-label, dose-escalation study evaluating the safety, tolerability, and pharmacokinetics of enzalutamide (previously MDV3100) alone or in combination with an aromatase inhibitor in women with advanced breast cancer. SABCS 2013 PD3-6 (A938), accepted, poster discussion.

Traina TA, Yardley, DA, Patel M, Schwartzberg L, Elias A, Gucalp A, Peterson AC, Hannah A, Gibbons J, Khondker Z, Hudis CA, LoRusso P. A phase 1 open-label, dose-escalation study evaluating the safety, tolerability, and pharmacokinetics of enzalutamide (previously MDV3100) alone or in combination with an aromatase inhibitor in women with advanced breast cancer. SABCS 2013 PD3-6 (A938), accepted, poster discussion.

Traina TA, Yardley DA, Patel MR, Schwartzberg LS, Elias A, Gucalp A, Blaney ME, Gibbons J, Hudis CA, LoRusso P. A phase 1 open-label study evaluating the safety, tolerability, and pharmacokinetics of enzalutamide alone or combined with an aromatase inhibitor in women with advanced breast cancer. IMPAKT 2014 Breast Cancer Conference May 2014, Abstract 214.

Schwartzberg LS, Yardley DA, Elias A, Patel MR, Gucalp A, Burris HA, Peterson AC, Hannah AL, Blaney ME, Gibbons J, Tudor IC, Steinberg JL, LoRusso P, Infante JR, Hudis CA, Traina TA. Enzalutamide plus exemestane: a pilot study to assess safety, pharmacokinetics, and effects on circulating estrogens in women with advanced hormone-positive breast cancer. Proc ASCO 2014.

Elias AD, Burris HA, Patel MR, Schwartzberg LS, Richer JK, Kavalerchik E, Stopatschinskaja S, Gibbons J, Markova D, Steinberg JL, Traina TA. MDV3100-08: a phase I study evaluating the safety and pharmacokinetics of enzalutamide plus fulvestrant in women with advanced hormone receptor-positive breast cancer. Proc SABCS 2015, accepted poster presentation.

D'Amato NC, Jacobsen BM, Cochrane DR, Spoelstra NS, Babbs BL, Elias A, Richer JK. Inhibiting androgen receptor nuclear localization decreases estrogen receptor (ER) activity and tumor growth in ER+ breast cancer. Proc SABCS 2014, P3-04-06

Gordon MA, D'Amato N, Gu H, Wong D, Elias A, Richer JK. Targeting multiple pathways in breast cancer: Androgen receptor, HER2, and mTOR. Proc SABCS 2014, P6-03-07.

Gordon MA, D'Amato N, Gu H, Liu B, Elias A, Richer JK. The anti-androgen enzalutamide synergizes with trastuzumab and everolimus to inhibit breast cancer growth via distinct mechanisms. Endocrine Society 2015.
Elias AD, Burris HA, Patel MR, Schwartzberg LS, Richer JK, Kavalerchik E, Stopatschinskaja S, Gibbons J, Markova D, Steinberg JL, Traina TA. MDV3100-08: a phase I study evaluating the safety and pharmacokinetics of enzalutamide plus fulvestrant in women with advanced hormone receptor-positive breast cancer. Proc SABCS 2015, accepted poster presentation.

Gordon MA, D'Amato N, Christenson J, Babbs B, Gu H, Wulfkuhle J, Petricoin E, Elias A, Richer JK. Synergy between androgen receptor (AR)-targeting agents seviteronel (SEVI) or enzalutamide (ENZA) and mTOR or HER2 pathway-targeting agents in breast cancer. Proc AACR 2017.

Targeting the Androgen Receptor with Seviteronel, a CYP17 Lyase and AR inhibitor, in Triple Negative Breast Cancer. Reese JM[1], Babbs BL[1], Christenson JL[1], Spoelstra NS[1], Elias A[2], Eisner JR[3], Baskin-Bey ES[3], Gertz J[4], and Richer JK. Targeting the Androgen Receptor with Seviteronel, a CYP17 Lyase and AR inhibitor, in Triple Negative Breast Cancer. SABCS 2018.

Williams MM[1], Spoelstra NS[1], Arnesen S [2], Christenson JL[1], Babbs BL[1], O'Neill, K[1], Reese JM[1], Blanchard ZD[2], Hanamura T[1], Elias A[3] , Gertz J[2], Jacobsen BM[1] and JK Richer. Unique response of *ESR1* mutant breast cancer to stress associated with disease progression. International Association of Breast Cancer Research April 2019 The Netherlands

Williams MM[1], Spoelstra NS[1], Arnesen S [2], Christenson JL[1], O'Neill, K[1], Reese JM[1], Blanchard ZD[2], Hanamura T[1], Jacobsen BM[1], Gertz J[2], Elias A[3] and JK Richer[1] Preclinical and Clinical Analyses of Estrogen Receptor Mutant Metastatic Breast Cancer. SABCS 2019.

Oral Presentations of Richer/Elias team work

Oct 2013 **AACR Advances in Breast Cancer Research Conference** Invited Symposia Lecture "Targeting the Androgen Receptor in Breast Cancer", Targeted Therapies San Diego, CA.

Jan 2014 **Keystone Symposia on Nuclear Receptors:** Biological Networks, Genome Dynamics and Disease. Invited short talk: Role of Androgen Receptors in Estrogen Receptor Negative Breast Cancer.Taos, NM.

March 2014 **Society for Gynecologic Investigation**. Invited Symposia Lecture "Functional Significance of miRNAs Contributing to Reproductive Cancers" Florence, Italy,

Sept 2015 **US Oncology Mckesson Annual Scientific Forum Invited Lecture** "Role of Androgen Receptors in Breast Cancer" Dallas, TX

Jan 2016 **Keystone Symposium Nuclear Receptors** Scientific organizer and speaker "Subtype-Specific AR Action in Breast Cancer" Snowbird, Utah.

April 2016 **Endocrine Society Annual Meeting 2016** Invited Symposia Lecture "Role of Androgen Receptors in Breast Cancers Resistant to Estrogen Receptor-Directed Endocrine Therapies" Boston, MA

May 2016 **Gordon Research Conference on Mammary Gland Biology and Cancer** Short talk "Targeting the Androgen Receptor in Triple Negative Breast Cancer, Lucca, Italy

June 2017 **FASEB SRC Meeting** on Rapid Signaling & Genomic Hormone Action in Health & Disease. Invited oral presentation on "Androgen Receptor and mTOR Cross Talk in Breast Cancer"

Oct 2017 **Breast Cancer Research Foundation** Think Tank for Androgen Receptor in Breast Cancer

Dec 2017 **San Antonio Breast Cancer Symposium**. Invited Educational Session presentation "Androgen Receptors in Breast Cancer" Symposium on Androgen, Progesterone and Glucocorticoid Receptors: Reprogramming of Steroid Receptors during Breast Tumor Progression.

April 2018 **AACR Invited "Meet the Expert" sessio**n "Update on Potential for Targeting Androgen Receptors in Breast Cancer."

Aug 2019 **Gordon Research Conference Hormones and Cancer**, Sunday River, Maine, "Hormone Deprivation Influences Breast Cancer Immune Suppression"

Website(s) or other Internet site(s)
List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Expert Opinion piece in Oncology PracticeUpdate http://www.practiceupdate.com/journalscan/9370 or http://prac.co/j/5960d32c-988b-423e-ba24-14ca5c8cc39a?elsca1=soc_share-this acknowledgement of federal support –no

Highlight of Cochrane DR et al Breast Cancer Research 2014 in Feb issue of 2014 NATURE REVIEWS CLINICAL ONCOLOGY. acknowledgement of federal support –yes

Personnel who received pay from the research effort

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Solem, Julie
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Talamantes, Sarah
Thor, Ann
Zolman, Katherine