AWARD NUMBER: W81XWH-18-1-0016

TITLE: Targeted Inhibition of Leukemia Inhibitory Factory (LIF)/LIFR Axis for the Treatment of Triple-Negative Breast Cancer

PRINCIPAL INVESTIGATOR: RATNA VADLAMUDI

CONTRACTING ORGANIZATION:
University of Texas Health Science Center at San Antonio
San Antonio, TX  78229

REPORT DATE: Feb 2019

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland  21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
Leukemia inhibitory factor receptor (LIFR) and its ligand LIF play a critical role in cancer progression. Here, we describe a rationally designed first-in-class inhibitor of LIFR, EC359 that directly interacts with LIFR and effectively block LIF/LIFR interactions. The results from first year studies showed that EC359 treatment exhibit anti-proliferative effects, reduce invasiveness and promote apoptosis of TNBC cells. The activity of EC359 is dependent on LIFR expression and treatment with EC359 attenuated the activation of LIF/LIFR driven pathways including STAT3, mTOR, and AKT. EC359 significantly reduced tumor progression in TNBC xenografts. EC359 exhibited good ADME characteristics. Collectively, these data support EC359 as a novel targeted therapeutic that inhibits LIFR oncogenic signaling that occur in TNBC.
Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Introduction</td>
<td>4</td>
</tr>
<tr>
<td>2. Keywords</td>
<td>4</td>
</tr>
<tr>
<td>3. Accomplishments</td>
<td>4</td>
</tr>
<tr>
<td>4. Impact</td>
<td>10</td>
</tr>
<tr>
<td>5. Changes/Problems</td>
<td>10</td>
</tr>
<tr>
<td>7. Participants &amp; Other Collaborating Organizations</td>
<td>12</td>
</tr>
<tr>
<td>8. Special Reporting Requirements</td>
<td>12</td>
</tr>
<tr>
<td>9. Appendices</td>
<td>13-15</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

Triple negative breast cancer (TNBC) has poor prognosis, lack targeted therapies, contain high percentage of cancer stem cells, and often exhibit therapy resistance. TNBC are more aggressive, and due to lack of targeted therapies, they represent a disproportional share of the breast cancer mortality. TNBC tumors are characterized by high propensity to metastasis, some subtypes of TNBC such as claudin-low are highly enriched with cancer stem cells and frequently exhibit therapy resistance. The propensity of TNBC to metastasize to visceral sites is another major clinical problem and identifying factors that contribute to metastases are needed for advancement of therapies. There is a major need for rationally designed novel therapies that can improve response to TNBC treatment and extend survival.

Leukemia inhibitory factor (LIF) is the most pleiotropic member of the interleukin-6 family of cytokines that signals via the LIF receptor (LIFR). LIF activate multiple signaling pathways including STAT3, MAPK, AKT, and mTOR that are implicated in TNBC progression. Recent evidence implicated tumors exhibit upregulated LIF-LIFR signaling via autocrine and paracrine mechanisms. LIF promotes proliferation and metastasis of breast cancer cells. TNBC cells have higher expression of LIF compared to ER-positive breast cancer cells and overexpression of LIF significantly associated with a poorer relapse free survival in breast cancer patients. LIF signaling play a role in crosstalk between tumor cells and fibroblasts and mediate pro-invasive activation of stromal fibroblasts. LIF-LIFR axis is implicated in the maintenance of stem cells and deregulation of LIF signaling contribute to chemoresistance. Together, these emerging findings strongly suggest that LIF signaling in TNBC may be clinically actionable and that disruption of the LIF signaling has potential to block TNBC progression.

With our expertise in developing novel small molecule inhibitors that disrupt protein-protein interactions and SAR studies using our compound libraries, we have rationally designed and synthesized a first-in class LIF inhibitor compound. The objective of this proposal is to confirm the specificity, test in vivo activity and establish the mechanism, and conduct safety, efficacy, off target screening studies. Our hypothesis is that LIFR signaling play a critical role in TNBC progression and disruption of LIF-LIFR interaction with small molecule inhibitor EC359 will have therapeutic effect.

2. KEYWORDS

Breast cancer; TNBC; LIFR; LIF; STAT3; xenograft; tumor progression;

3. ACCOMPLISHMENTS

During the first 12 months of the project, from February 1, 2018 to January 31, 2019, we have made significant progress in the proposed experiments as detailed in section 3.2 and are on schedule to complete the remaining experiments. Major accomplishments include

- Completion of large scale synthesis of EC359 (Aim 3)
- Completion of ADME studies (Aim 3)
- Completion of biophysical and specificity experiments (Aim 2)
- Completion of signaling studies (Aim 2)
- Completion of in vitro assays confirming the EC359 on various TNBC models (Aim 1)
- Completion of one Xenograft study and initiation of additional xenograft studies (Aim 1)

3-1. What were the major goals of the project?

Aim 1. To establish the efficacy of EC359 using preclinical, syngeneic and PDX models of TNBC.

Aim 2. To establish the molecular mechanism of action of EC359 and to identify potential biomarkers.

Aim 3. To establish ADME, pharmacokinetics (PK), and toxicology of EC359.
3-2. What was accomplished under these goals?

**Major task 1: To establish the efficacy of EC359 using preclinical, syngeneic and PDX models of TNBC.**

**Subtask 1: To characterize the biological activity of EC359 in vitro (Time line 1-12 months)**

EC359 reduced the cell viability of LIF and LIFR expressing cells. We first examined the expression of LIF and LIFR in cells that represent various subtypes of TNBC (BT-549, SUM-159, MDA-MB-231, HCC1937, MDA-MB-468, and HCC1806), ER+ve breast cancer (MCF7, and T47D) as well as normal mammary epithelial cells (HMEC). We found that five of the six TNBC cells expressed high levels of LIF and LIFR when compared to ER+ve breast cancer cells and normal cells (Figure 1A,B). Next, we examined the efficacy of EC359 on cell viability of TNBC and ER+ve breast cancer cells. Treatment with EC359 resulted in a significant dose-dependent reduction in the cell viability of TNBC cells (IC50 10-50 nM) and their inhibition is correlated well with the LIF and LIFR expression levels (Figure 1C). Interestingly ER+ve breast cancer cells which express low levels of LIF and LIFR exhibited low sensitivity to EC359 treatment (IC50 >1000 nM) when compared with TNBC cells (Figure 1D).

EC359 reduced invasion, and induced apoptosis of TNBC cells. We next examined the efficacy of the EC359 on the survival of TNBC cells. In clonogenic survival assays, EC359 significantly reduced the colony formation ability of MDA-MB-231 and SUM-159 cells (Figure 2A). Given the important role of the LIF axis in the invasiveness of cancer cells, we examined the effect of EC359 in reducing the invasion of TNBC cells. Matrigel invasion assays demonstrated that EC359 significantly reduced the invasion potential of MDA-MB-231 and BT-549 cells (Figure 2B). Further, we examined whether EC359 induced apoptosis in TNBC cells using caspase 3/7 activity assays. EC359 significantly increased the caspase 3/7 activity in MDA-MB-231 and BT-549 cells (Figure 2C). Collectively, these results suggest that EC359 exhibits significant inhibitory activity on invasion and promotes apoptosis of TNBC cells.

**Multi-PI contributions:** The above work was jointly performed by Dr. Vadlamudi and Dr. Nickisch labs. Dr. Nickisch lab generated needed EC359, and tested its purity and activity. Dr. Vadlamudi lab characterized biological activity using TNBC models.

**Subtask 2: Test the efficacy of EC359 on blocking TNBC tumorigenesis in vivo (Time line 6-24 months)**

IACUC and ACURO Approval: Approvals have been obtained and these experiments were ongoing

EC359 reduced TNBC xenograft tumor growth in vivo: To test the efficacy of EC359 on in vivo tumor progression,
we established MDA-MB-231 xenograft tumors in the mammary fat pad of nude mice. Mice were randomized to vehicle (hydroxy methyl cellulose) and EC359 (5 mg/kg/day via subcutaneous injection) 3 days/week. EC359 treatment significantly reduced the tumor progression compared to vehicle (Figure 3A). Mice body weights in the vehicle and EC359 treated groups were similar (Figure 3B) confirming the low toxicity of EC359. Further, EC359 treated tumors exhibited fewer proliferating cells (Ki-67 positive cells) compared to vehicle treated tumors (Figure 3C). Further, RTqPCR analysis confirmed significant decrease in the activation of STAT3 target genes in EC359 treated tumors compared to control (Figure 3D). Western blot analysis confirmed that xenograft tumors express LIFR and LIF (Figure 3E). Further, EC359 treatment substantially reduced the phosphorylation of STAT3 and ERK1/2 pathways in EC359 treated tumors compared to vehicle treated tumors (Figure 3E). Collectively, these results suggest that EC359 has potent anti-tumor activity on TNBC in preclinical models.

**Multi-PI contributions:** The above work was jointly performed by Dr. Vadlamudi and Dr. Nickisch labs. Dr. Nickisch lab synthesized needed EC359 compound and optimized process development. Dr. Vadlamudi’s lab conducted xenograft studies, tumor measurement and biochemical characterization.

**Subtask 3: Effect of EC359 on blocking TNBC tumor progression to metastasis (Time line 12-24 months)**
These studies will be initiated during second year

**Subtask 4: Test the efficacy of EC359 combination therapy in reducing the growth of therapy resistant TNBC in vivo (Time line 24-36 months)**
These studies will be initiated during third year

**Subtask 5: Test the efficacy of EC359 in reducing the growth of TNBC PDX tumors in vivo (Time line 18-36 months)**
These studies will be initiated during second year

**Major Task 2: To establish the molecular mechanism of action of EC359 and to identify potential biomarkers**

**Subtask 1: Determine the specificity of EC359 for targeting LIFR (Time line 1-12 months)**
Surface plasmon resonance (SPR) studies confirmed EC359 direct interaction with LIFR. To test whether EC359 directly binding to LIFR complex, binding profiles of EC359 to LIF/LIFR were evaluated using surface plasmon resonance (SPR). Two sets of studies were performed: 1) to verify the integrity of recombinant proteins, the interaction between LIFR and LIF was studied; 2) small molecule binding to LIF/LIFR by either immobilizing LIFR or LIF onto a sensor chip was tested. Results from the first set of studies confirmed the integrity of recombinant LIF and LIFR; LIF bound to immobilized LIFR-Fc with a binding constant of 7µM (Figure 4A). In the second set of studies, results showed EC359 binding to LIFR, but not LIF. Further, EC359 bound to LIFR in a dose dependent manner with KD 81µM (Figure 4B). The results confirmed that EC359 is a specific inhibitor of LIF/LIFR complex.

---

**Figure 3.** A, MDA-MB-231 xenografts (n=7) were treated with vehicle or EC359 (5mg/kg/s.c./3 days/week). Tumor volumes are shown in the graph. B, Body weights of vehicle and EC359 treated mice are shown. C, Ki-67 expression as a marker of proliferation was analyzed by IHC and quantitated. D, Status of STAT3 target genes were measured by using RT-qPCR analysis (n=3). E, LIFR downstream signaling was measured using western blotting (data using two different xenograft tumors is shown). * P<0.05, ** P<0.01, *** P<0.001, ****p<0.0001.

**Figure 4.** SPR studies. A, Confirmation of functionality of LIF/LIFR interaction. B, Confirmation of LIFR-EC359 interaction.
Microscale thermophoresis (MST) assays revealed high affinity interaction of EC359 with LIFR. Since SPR studies use immobilized receptor complex, we conducted an orthogonal assay where receptor is not immobilized to verify EC359 binding to the receptor complex. MST is a powerful technique to quantify biomolecule interactions. By combining the precision of fluorescence detection with the variability and sensitivity of thermophoresis, MST provides a flexible, robust and fast way to dissect molecular interactions. MST analysis confirmed direct interaction of EC359 with LIFR with an estimated KD of 10.2 nM (Figure 5).

CRISPR KO cells confirmed requirement of LIFR for EC359 function. To further confirm the target specificity of EC359, we generated doxycycline inducible LIFR-KO cells using Cas9 stably expressing TNBC cells. Results indicated a reduction of LIFR expression in BT-549 models contributed to the resistance of the EC359 mediated decrease in cell viability (Figure 6). Collectively, this data suggests that EC359 activity depends on presence of functional LIF/LIFR signaling axis in cells.

**Multi-PI contributions:** The above work was jointly performed by Dr. Vadlamudi and Dr. Nickisch labs. Dr. Nickisch lab completed biophysical studies charactering EC359 interaction with LIFR including SPR and MST studies.

**Subtask 2: Test the effect of EC359 on LIF-LIFR signaling**

EC359 reduced LIFR mediated activation of downstream signaling pathways. To further confirm the effect of EC359 on LIF/LIFR downstream signaling pathways, MDA-MB-231 and BT-549 cells were pretreated with vehicle or EC359 and subsequently stimulated with LIF. STAT3 activation was examined using western blotting. EC359 treatment substantially reduced the LIF activation of STAT3 in both BT-549 and MDA-MB-231 cells (Figure 7A). Further, EC359 treatment also substantially decreased the phosphorylation of AKT, mTOR, S6 and ERK1/2 in MDA-MB-231 and BT-549 cells (Figure 7B,C). EC359 treatment also increased the phosphorylation of proapoptotic p38MAPK in BT-549 cells (Figure 7C). These results suggest that EC359 acts as a LIFR inhibitor and attenuates LIFR mediated signaling in TNBC cells.

**Multi-PI contributions:** The above work was jointly performed by Dr. Vadlamudi and Dr. Nickisch labs. Dr. Nickisch lab synthesized needed EC359 and optimized process development. Dr. Vadlamudi’s lab conducted signaling and western studies. Both labs collectively interpreted the data.

**Subtask 3: Test the effect of EC359 on TNBC signaling pathways. (Time line 1-24 months)**

We have initiated these studies. Pathways and bioinformatic analysis is ongoing and will be completed in second year.

**Subtask 4: Test the Effect of EC359 on TNBC Stemness (Time line 12-36 months)**

These studies will be initiated during second year and will continued in third year.
Major Task 3: To establish ADME, pharmacokinetics (PK), and toxicology of EC359

Subtask 1: To synthesize EC359 in large quantities.

The specific aim for the chemistry part involved the synthesis of enough quantities of EC359 for preclinical *in vitro* as well as *in vivo* studies. During first year, we were able to develop a process for the synthesis of EC-359 so that large quantities can be synthesized efficiently.

The medicinal chemistry route for the preparation of EC-359 is shown in Scheme 1.

![Scheme 1](image)

The medicinal chemistry route consists of 7 steps and involve 5 chromatographic purifications. Moreover, it’s lengthy, it involves the use of heavy metal such as palladium in the main synthetic scheme. The need for multiple chromatographies along the synthesis makes it very costly. The overall yield for EC-359 using the medicinal chemistry route was found to be only 28%.

To summarize the drawbacks of the above synthesis:

1. Long synthesis
2. Involve the use of a heavy metal such as palladium
3. Multiple chromatographies for purification.

Several synthetic routes were designed and tested for addressing the drawbacks and the route representing the most efficient and viable set of reactions are represented in scheme 2.
Compound Assays Result

EC359 Mutagenicity testing - S. typhimurium TA98, TA100, TA1535 and E.coli WP2 uvrA + E. coli WP2uvrA (hIGR) strains
No mutagenicity

EC359 Cardiotoxicity assessment (hERG): IC50 against HEPG membrane using a fluorescence polarization assay
No liability

EC359 CYP inhibition: In vitro evaluation of CYP3A4 activity was tested using human liver microsomes (1A2, 2C9, 2C19, 2D6, 3A4)
2D6 inhibition

EC359 Hepatocyte stability: In vitro evaluation of EC359 compound for metabolic stability using cryopreserved human, mouse, rat and dog hepatocytes
Human & Mouse-moderate

EC359 Microsomal stability: In vitro evaluation of EC359 compound for metabolic stability using cryopreserved human, mouse, rat and dog liver microsomes
Human & Mouse-moderate

EC359 Plasma-protein binding (%) Human: 99.89; Mouse: 99.63; Rat: 99.98; Dog: 99.83

EC359 Plasma stability (% remaining at 80 min) Human: 100.64; Mouse: 105.61

EC359 Solubility (µg/mL) Low: <10 µg/mL

EC359 Toxicity assessment (oral toxicity, dermal toxicity, and inhaled toxicity) None

EC359 Metabolism stability: Major metabolic pathway: Phase I metabolism; No glucuronide metabolites (as evidenced by low CL in UDPGA

EC359 Caco2-permeability & efflux transporter substrate activity Low permeability; No efflux transporter substrate activity

EC359 HTS of LIF and LIFR binding - Thermophoresis method Kd LIFR: 10.24; Kd LIF: No binding up to 5µM

Subtask 2: To establish Absorption, Distribution, Metabolism, Excretion (ADME) of EC359 (Time line 1-24 months)

During the first year, we have completed ADME studies using CRO and results are summarized in figure 8. Ames test confirmed that EC359 did not induce an evident (significant) >2 fold increase in the revertant counts at the doses tested (dose related), with the tester strains both with and without metabolic activation according to the evaluation criteria mentioned in OECD guideline no.471. Hence, the compound EC359 is considered non-mutagenic with salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and E.coli combo, both with and without metabolic activation. Among CYP enzymes EC359 inhibits 2D6 hence caution is warranted in concurrent administration that inhibits 2D6 such as Prozac. Metabolic stability and plasma stability are moderate in human with high plasma protein binding (Figure 8). Collectively, the data from these studies indicate EC359 as a druggable candidate for further development after establishing toxicology and safety pharmacology studies.

Multi-PI contributions: The above work was performed by Drs. Nickisch lab and Vadlamudi lab. Synthesis was done in Dr. Nickisch lab and the biological activity was tested in Vadlamudi lab.

Subtask 3: Pharmacokinetic (PK) studies of EC359 in vivo (Time line 18-30 months)

These studies will be initiated during second year.
Subtask 4: MTD and Toxicology Studies for EC359 (Time line 25-36 months)

These studies will be initiated during late second year and completed in third year

3-3. What opportunities for training and professional development has the project provided?

During the first year, this project provided training opportunities for four students (one graduate student, one undergraduate student, one post-doctoral fellow and one high school student). All the students/fellows were trained in conducting breast cancer research using BCa model cells, preclinical animal models, designing/analyzing research experiments and interpreting the data. In addition, Post-doctoral fellow was given opportunity to train graduate and undergraduate students, this provided an opportunity to sharpen her mentoring skills. Graduate students were provided an opportunity to serve as mentor of high school student. All students were participated in weekly project meetings and presented their research once in a month as oral presentation. In addition, students/fellows were provided several professional development opportunities including participation in journal clubs, attendance of grand rounds, and attendance of ethics seminar series. Further, post doc and graduate student attended 2018 AACR SABCS annual meeting to present the research findings

3-4. How were the results disseminated to communities of interest?

The results were presented as a poster presentation in Dec 2018 AACR-SABCS annual meeting. Another poster presentation is scheduled in April 2019 AACR annual meeting.

A manuscript deataining the first year results was being submitted as full manuscript to Molecular Cancer Therapeutics Journal.

3-5. What do you plan to do during the next reporting period to accomplish the goals?

We will start performing the following tasks during second year:
Main task 1; Subtask 3: Effect of EC359 on blocking TNBC tumor progression to metastasis
Main task 1: Subtask 5: Test the efficacy of EC359 in reducing the growth of TNBC PDX tumors in vivo
Main Task 2; Subtask 3: Test the effect of EC359 on TNBC signaling pathways.
Main Task 2; Subtask 4: Test the Effect of EC359 on TNBC
Main Task 3; Subtask3: Pharmacokinetic (PK) studies of EC359
Main Task 3; Subtask4: MTD and Toxicology Studies for EC359

4. IMPACT

4-1. What was the impact on the development of the principal discipline(s) of the project?

Our data demonstrated that EC359 is a highly potent and LIFR specific inhibitor. EC359 blocked LIF/LIFR physical and functional interaction, signaling and reduced cell viability of LIF/LIFR expressing TNBC cells both in vitro and in vivo. EC359 represents an exciting new mechanism to modulate LIF/LIFR oncogenic functions. Since EC359 is a small, stable molecule, it is amenable for translation to clinical trials. However, additional preclinical studies proposed in year 2 and 3 are needed to completed to fully understand EC359 potential.

4-2. What was the impact on other disciplines?
LIF and LIFR are over-expressed in multiple solid tumors. While LIF can act on a wide range of cell types, LIF knockout mice have revealed that many of these actions are not apparent during ordinary development, indicating a potential therapeutic window for LIF/LIFR axis inhibitors and less toxicity in normal adult tissues. Considering the wide deregulation of LIF/LIFR axis in multiple tumors, small molecule LIFR inhibitor EC359 may have utility in treating other solid tumors including glioblastoma, ovarian cancer, colon cancer, and pancreatic cancer all which exhibit deregulated LIF/LIFR signaling.

4-3. What was the impact on technology transfer?
Nothing to Report.

4-4. What was the impact on society beyond science and technology?
Nothing to Report.

5. CHANGES/PROBLEMS
5-1. Changes in approach and reasons for change.
Nothing to Report.

5-2. Actual or anticipated problems or delays and actions or plans to resolve them
The project is going well with no anticipated problems or delays. If our time permits, we intend to early start Subtask4: MTD and Toxicology Studies for EC359 studies (originally proposed in Year 3) during the latter part of second year

5-3. Changes that had a significant impact on expenditures
Nothing to Report.

5-4. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to Report.

6. PRODUCTS
6-1. Publications, conference papers, and presentations


6-2. Website(s) or other Internet site(s)
6-3. Technologies or techniques
Nothing to Report.

6-4. Inventions, patent applications, and/or licenses
Nothing to Report.

6-5. Other Products
Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

7-1. What individuals have worked on the project?

<table>
<thead>
<tr>
<th>Name</th>
<th>Project Role</th>
<th>Effort (mo.)</th>
<th>Site</th>
<th>Contribution to Project</th>
<th>Other Funding Support and Changes in the Reporting Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Klaus Nickisch</td>
<td>Initiating PI</td>
<td>1.2</td>
<td>Evestra</td>
<td>Experiment design, project supervision, data analysis, Partner PI coordination, data presentation</td>
<td>Active: None to report</td>
</tr>
<tr>
<td>Bindu Santhamma</td>
<td>Co-investigator</td>
<td>5.4</td>
<td>Evestra</td>
<td>Chemical synthesis, process development</td>
<td>Active: None to report</td>
</tr>
<tr>
<td>Migdalis Cintron-Miguel</td>
<td>Co-Investigator</td>
<td>2.94</td>
<td>Evestra</td>
<td>Analytical method development, analytical measurements</td>
<td>Active: None to report</td>
</tr>
<tr>
<td>Gulzar Ahmed</td>
<td>Co-Investigator</td>
<td>1.8</td>
<td>Evestra</td>
<td>Chemical synthesis, conjugation chemistry</td>
<td>Active: None to report</td>
</tr>
<tr>
<td>Rebecca Huff</td>
<td>Administrative assistant</td>
<td>0.48</td>
<td>Evestra</td>
<td>Budgetary support</td>
<td>Active: None to report</td>
</tr>
<tr>
<td>Angel Alcala</td>
<td>Administrative assistant</td>
<td>1.2 months</td>
<td>Evestra</td>
<td>Logistical support</td>
<td>Active: None to report</td>
</tr>
<tr>
<td>Ratna Vadlamudi</td>
<td>Partner PI</td>
<td>1.8</td>
<td>UTHSCSA</td>
<td>Experiment design, project supervision, data analysis, Partner PI coordination, data presentation</td>
<td>Active: R01CA178499 (2.4 mo); R01CA179120-01A1 (0.96 mo); R01CA223828 (1.56 mo); R01NS088058-01A1 (0.96 mo); DOD BC151884 (0.48 mo); DOD BCRP: W81XWH-18-1-0021 (0.6 mo)</td>
</tr>
<tr>
<td>Suryavathi Viswanadhapalli</td>
<td>Post-doctoral fellow</td>
<td>9</td>
<td>UTHSCSA</td>
<td>Experiment design and execution, data analysis and presentation</td>
<td>Active: DOD BCRP :W81XWH-18-1-0021 (3 mo)</td>
</tr>
<tr>
<td>Xaionan Li</td>
<td>Research Assistant</td>
<td>4.8</td>
<td>UTHSCSA</td>
<td>Experiment execution, data analysis and presentation</td>
<td>Active: R01CA223828 (6 mo)</td>
</tr>
</tbody>
</table>
7-2. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
A minor change in one co-investigator. Since James Goebel left Evestra, we have replaced him with Migdalis Cintron-Miguel, an equally qualified person. Cintron-Miguel has extensive experience and expertise in analytical chemistry in pharmaceutical industry.

7-3. What other organizations were involved as partners?
Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS
8-1. COLLABORATIVE AWARDS
This Progress Report is jointly authored by Dr. Klaus Nickisch (Initiating PI) and Dr. Ratna Vadlamudi (Partner PI) and provides details for the work performed in their respective laboratories. An identical copy of this Report will be submitted by Dr. Klaus Nickisch and Dr. Vadlamudi independently as their respective Progress Report.

8-2. Quad Chart
Not applicable.

9. APPENDICES
Reprints of Abstracts included on page 14, 15
Development of a first-in-class small molecule inhibitor (EC359) targeting oncogenic LIF/LIFR signaling for the treatment of triple negative breast cancer


**Room:** Hall 1

**Date/Time:** Thursday, December 6, 2018 - 7:00 am

**Session Info:** Poster Session 2: Tumor cell and molecular biology: Novel/Emerging Therapeutic Targets (7:00 AM-9:00 AM)

**Background:** Leukemia inhibitory factor (LIF) and its receptor LIFR are over-expressed in multiple solid tumors and play a key role in tumor growth, progression, and resistance to standard anti-cancer treatments. Triple-negative breast cancer (TNBC) lacks targeted therapies and represents a disproportional share of breast cancer (BCa) mortality. TNBC exhibits autocrine stimulation of the LIF/LIFR axis and overexpression of LIF is associated with poorer relapse-free survival in BCa patients. LIF signaling also promotes maintenance of stem cells. Therefore, targeting the LIF/LIFR axis may have therapeutic utility in TNBC.

**Methods:** We rationally designed a small organic molecule (EC359) that emulates the LIF/LIFR binding site and functions as a LIFR inhibitor from a library of compounds. *In silico* docking studies were used to identify the putative interaction of the EC359 and LIF/LIFR complex. Direct binding of EC359 to LIFR was confirmed using surface plasmon resonance (SPR) and microscale thermophoresis technique (MST) assays. *In vitro* activity was tested using Cell-Titer Glo, MTT, invasion, and apoptosis assays. Mechanistic studies were conducted using Western blot, reporter gene assays, and RNA-seq analysis. Xenograft, patient-derived xenograft (PDx), and patient-derived explant (PDEX) models were used for preclinical evaluation and toxicity.

**Results:** Molecular docking studies showed that EC359 interacts at the LIF/LIFR binding interface. SPR and MST studies confirmed direct interaction of EC359 to LIFR. EC359 reduced the growth of TNBC cells with high potency (IC50 50-100nM) and promoted apoptosis. Further, EC359 treatment reduced invasion and stemness of TNBC cells. EC359 activity is dependent on the expression levels of LIFR and showed little or no activity on TNBC cells that have low levels of LIFR or ER+ve BCa cells. Further, EC359 significantly reduced the viability of cisplatin and taxane-resistant TNBC cells and enhanced the efficacy of HDAC inhibitors. Mechanistic and biochemical studies showed that EC359 interacts with LIFR and effectively blocking LIF/LIFR interactions. EC359 also blocked LIFR interactions with other LIFR ligands such as oncostatin M, ciliary neurotrophic factor, and cardiotrophin-1. EC359 treatment attenuated the activation of LIF/LIFR driven pathways including STAT3, mTOR, AKT, and MAPK. RNA-seq analysis identified regulation of apoptosis as one of the important pathway modulated by EC359. In TNBC xenograft and PDX assays, EC359 significantly reduced tumor progression. Further, using human primary BCa PDEX cultures, we demonstrated that EC359 has the potential to substantially reduce the proliferation of human BCa. Pharmacologically, EC359 exhibited high oral bioavailability and long half-life with a wide therapeutic window.

**Conclusions:** EC359 is a novel targeted therapeutic agent that inhibits LIF/LIFR oncogenic signaling in TNBC via a unique mechanism of action. EC359 has the distinct pharmacologic advantages of oral bioavailability, *in vivo* stability, and is associated with minimal systemic side effects. (DOD BCRP grant #BC170312)
Therapeutic utility of EC559 for targeting oncogenic LIFR signaling in triple negative breast cancer

Short Title: LIFR therapy for breast cancer

Author Block: Suryaviniti Viswanadhapalli\textsuperscript{1}, Mengxing Li\textsuperscript{1}, Yiliao Luo\textsuperscript{1}, Gangadhar Reddy\textsuperscript{1}, Bindu Santanam\textsuperscript{2}, Mei Zhou\textsuperscript{3}, Shihong Ma\textsuperscript{4}, Rajni Sonavane\textsuperscript{5}, Jyotsa P. Pratap\textsuperscript{6}, Kristin A. Allweg\textsuperscript{7}, Anandh Chang\textsuperscript{8}, Alejandra Chávez-Riveros\textsuperscript{9}, Katarzak V. Dileep\textsuperscript{6}, Kam Y. Zhang\textsuperscript{10}, Marek Bajda\textsuperscript{11}, Ganeel V. Rai\textsuperscript{12}, Andrew Brunner\textsuperscript{13}, Vishaya Mantchi\textsuperscript{14}, Manjeet Redi\textsuperscript{15}, Rajeshwar R. Tekmal\textsuperscript{16}, Harshesh S. Nair\textsuperscript{17}, Klaus J. Nicklisch\textsuperscript{18}, Ratna K. Yadavamdi\textsuperscript{11}, UT Health Science Ctr., et San Antonio, San Antonio, TX; \textsuperscript{2}Evestra, Inc, San Antonio, TX; \textsuperscript{3}Second Xiangya Hospital, San Antonio, TX; \textsuperscript{4}UT Southwestern, Dallas, TX; \textsuperscript{5}Instituto de Química, Mexico; \textsuperscript{6}Center for Biosystems Dynamics Research, RIKEN, Yokohama, Japan; \textsuperscript{7}Jagellonian University Medical College, Cracow, Poland; \textsuperscript{8}Greenery Children’s Cancer Research Institute, San Antonio, TX

Abstract: Background: Leukemia inhibitory factor receptor (LIFR) and its ligand LIF play a major critical role in cancer progression, metastasis, stem cell maintenance, and therapy resistance. Recent studies in breast cancer have shown that feedback activation of LIFR limits response to histone deacetylase (HDAC) inhibitors and induce resistance. We rationally designed a small molecule (EC559) that antagonizes the LIF-LIFR binding site and functions as a LIFR inhibitor from a library of compounds. Here, we tested the utility of EC559 as a monotherapy and to effectively block LIF-LIFR interactions in overcoming resistance to HDAC inhibitors.

Methods: We have used multiple triple negative breast cancer (TNBC) models that represent all six types of TNBC. In vitro activity was tested using Cell-Titer Glo, MITT, Invasion, and apoptosis assays. Mechanistic studies were conducted using western blot, reporter gene assays, and RNA-seq analysis. Xenograft, patient-derived xenograft (PDX), and patient-derived explant (PDoX) models were used for preclinical evaluation and toxicity.

Results: EC559 treatment exhibited anti-proliferative effects, reduced invasiveness and stemness, and promoted apoptosis in all six TNBC cell lines. The activity of EC559 is dependent on LIF and LIFR expression. CRISPR-mediated knockdown of LIFR significantly abolished EC559 activity. Treatment with EC559 attenuated the activation of LIFR-LIFR driven pathways including STAT3, mTOR, and AKT. EC559 significantly reduced tumor progression in TNBC xenografts, PDX models, and reduced proliferation in patient-derived primary TNBC explants. In MITT based cell viability assays, addition of EC559 enhanced efficacy of SAHA compared to monotherapy of SAHA. In coculture survival assays, EC559 significantly enhanced efficacy of SAHA to reduce the colony formation compared to monotherapy. Mechanistic studies using three different TNBC models using western blot analysis and reporter gene assays confirmed activation of LIFR signaling pathway upon SAHA treatment and its blockage by EC559. Treatment of TNBC PDX explants with EC559 enhanced efficacy of SAHA to substantially decrease the proliferation (Ki-67 positivity) compared to monotherapy-treated tumors.

Conclusions: Collectively, these data support EC559 as a novel targeted therapeutic that inhibits LIFR oncogenic signaling as a monotherapy or in combination with HDAC inhibitors.

Author Disclosure Information:


Keywords/Indexing (Complete): Breast cancer; Histone deacetylase inhibitor; Small molecule inhibitor; Signal transducers and activators of transcription (STAT)

Financial Support for Attendance (Complete): Submission Fee (Complete): Your credit card order has been processed on Wednesday, 14 November 2018 at 11:16 PM. Status: Complete

***To log out, simply close your browser window. All information will be saved if you hit the Continue button after each step.

For all log-in problems or technical questions, please contact the OASIS Helpdesk or call (271) 398-1792.