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TITLE: Targeting Fatty Acid Synthase: A mechanism-guided approach to develop a novel therapeutic intervention for drug-resistant breast cancer

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CONTRACTING ORGANIZATION: **Mayo Clinic, Rochester, MN**

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14. ABSTRACT
Resistance to trastuzumab and HER2-directed therapy remains an unmet clinical need for patients with HER2+ breast cancer, and currently there are no FDA-approved drugs that can reverse resistance to trastuzumab or other HER2-directed therapies. Our preliminary data show that Fatty Acid Synthase (FASN) plays a major role in the maintenance of an aggressive breast cancer phenotype, and that FASN inhibition reduces tumor growth and augments the cytotoxicity of trastuzumab and paclitaxel. In this proposal we will evaluate TVB-2640, a FASN inhibitor that targets cancer metabolism and inhibits breast cancer growth. We will conduct a phase II trial of TVB-2640 in combination with paclitaxel and trastuzumab in patients with metastatic breast cancer who have disease resistant to trastuzumab. We will evaluate the safety and clinical efficacy of TVB-2640, as well as the value of serum and tissue FASN as novel biomarkers of response in HER2+ breast cancer.

15. SUBJECT TERMS:
Breast cancer, Trastuzumab, Paclitaxel, HER2, Fatty Acid Synthase (FASN), TVB-3199, Cancer metabolism, Drug resistance, Apoptosis, Biomarkers

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1.**INTRODUCTION:**

The development of HER2-targeted therapies has altered the natural course of HER2+ metastatic breast cancer (MBC) with a more favorable trajectory. The monoclonal HER2-directed antibody, trastuzumab (Trz), in combination with taxane-based chemotherapy such as paclitaxel (PXL) has an established clinical benefit for the treatment of HER2+ MBC. However, resistance inevitably ensues even for those with initial response, and novel approaches to overcome Trz-resistance remain an unmet clinical need. ***No FDA-approved drug that reverse resistance to trastuzumab (Trz) or other HER2-directed therapies*** **are currently available.**

Our preliminary data show that Fatty Acid Synthase (FASN) plays a major role in the maintenance of an aggressive BC phenotype. FASN inhibition interferes with BC tumor growth and augments the cytotoxicity of Trz and PXL, indicating that its inhibition has a chemo-sensitizing effect in BC. Most importantly, this is also true *in vivo* as FASN inhibition reduces tumor volume and synergizes with Trz in Trz-resistant, HER2+ BC xenograft models. ***Extending upon our prior studies of FASN and its role in tumor progression and response to therapy, we aim to develop novel, rationally designed therapeutic approaches for BC.***

In this proposal we will evaluate a potentially revolutionary BC therapy, TVB-2640, that targets cancer metabolism and inhibits BC growth in part through induction of cellular apoptosis. Resistance to standard therapies further stimulates BC progression, and our preclinical work suggests TVB-2640 can overcome Trz- and PXL-resistance in HER2+ BC models. ***We will conduct a phase II trial of TVB-2640 in combination with PXL and Trz in patients with breast cancer who have disease resistant to Trz. We will evaluate the clinical efficacy of TVB-2640, as well as the value of serum and tissue FASN as novel biomarkers of response in HER2+ BC.***

2. **KEYWORDS:**

Breast cancer
Trastuzumab
Paclitaxel
HER2
Fatty Acid Synthase (FASN)
TVB-2640
Cancer metabolism
Drug resistance
Clinical Trial
Biomarkers
Venetoclax (ABT-199)
Nnavitoclax (ABT-263).

3. ACCOMPLISHMENTS:
3.1. What were the major goals of the project?

Specific Aim 1: ***To assess the clinical activity of a novel FASN inhibitor, TVB-2640, in combination with paclitaxel and trastuzumab in a phase II clinical trial of patients with HER2+ metastatic breast cancer resistant to taxane and HER2-directed therapy.***

Specific Aim 2: ***To examine the clinical value of serum and tissue FASN expression as a novel theranostic marker in HER2+ breast cancer.***

Aim 1 and 2 are under the direction of Dr. Tufia Haddad. Please see separate annual progress report for details related to Specific Aims 1 & 2.

Specific Aim 3: **Specific Aim 3: Determine the mechanistic link between FASN inhibition-induced Bcl-2 pro-apoptotic BH3-only proteins and develop preclinical models in PDX mice based on targeting FASN and bcl-2.**

Major Task 3: Preclinical assessment of the FASN inhibitor TVB-3166 in combination with ABT263: Subtask C: The BEAUTY clinical trial: Preclinical assessment of the FASN inhibitor TVB-3166 (the form of TVB-2640 for animal use) in combination with Nnavitoclax, a Bcl2 and Bclxl inhibitor (ABT-263), [Alternatively we will use Venetoclax, a selective BCL2 inhibitor (ABT-199)] in patient-derived tumor xenografts: We hypothesize that inhibition of FASN re-sensitizes BC cells to the Bcl-2 pro-apoptotic pathway to induce cell death, and that dual blockade of anti-FASN (by TVB-3166) in combination with an anti Bcl-2 (by ABT-263) would result in a synergistic anti-tumor effect. ABT-263 synergistically induce anchorage independent growth and apoptosis of FASN expressing BC cells.

We have also shown to inhibition of FASN sensitizes tumors to PXL based therapies and anti-HER2 therapies. Thus, we will design studies to resensitize tumor to either PXL or Trz. To do so, we will utilize patient-derived xenografts (PDX), thereafter referred to as avatars. Avatars mice engrafted with viable tumor from consenting patients. The advantage of the avatars is that they more accurately recapitulate the individuality of human cancers and allow better prediction of response to therapy. Thus, avatar-directed therapy is projected to guide treatment selection, improve treatment response rates, and minimize delivery of non-effective agents to patients. Since we are working on the premise that inhibition of FASN chemosensitises tumors to chemotherapeutic and biological drugs, we will test at this point any advanced BC molecular –subtype, post chemotherapy. All established PDXs will be initially tested for FASN expression (IHC) Treatment and clinicopathologic outcomes from the source patients will be correlated in a de-identified fashion to these parameters. We have access to two different sources of PDX mice:

The BEAUTY clinical trial: For an immediate start, we have been granted access to 12 PDX models of HER2+ tumors (10 PDX mice that are Trz/taxane-sensitive and 2 PDX mice that are Trz/taxane-resistant) through the recently completed BEAUTY clinical trial. For all the studies we will need to supplement mice with estradiol and/or Tamoxifen and the combination of both. Additional Avatar models of the HER2+ subtype is available through the BEAUTY trial, and we are currently gathering all the necessary information regarding the availably and variety of these existing mice. The proposed experiments are as follows:

- The first experiment will address whether targeting FASN using TBV-3166 inhibits tumorigenicity. Mice will be randomized to each of the following treatment groups when their tumors have reached a size of 100 mm3: sterile vehicle TVB-3166 30mg/kg/day/iM, and TVB-3166 60mg/kg/day/iM. Mice will be sacred when the tumor reaches a size no higher than 2.0 cm2 or appears in distress. For each dose level of TBV-3166, the difference in the rate of change in tumor size (from pre-treatment to the time of sacrifice) with TBV-3166 and the rate of change in tumor size with vehicle will be assessed either using a two-sample t-test

or a Wilcoxon rank sum test. The dose level that differs most from vehicle will be carried forward into the next experiment.

- The second experiment will address whether adding a Bcl2 (ABT263) inhibitor add to TBV-3166's ability to inhibit tumorigenicity. Mice will be randomized to each of the following treatment groups when their tumors have reached a size of 100 mm 3: TVB-3166 dose from experiment 1 (TD1), TD1+ Nnavitoclax (150 nM) and TD1+ABT263 (300 nM). Mice will be sacred when the tumor reaches a size of 2.0 cm2 or appears in distress. For each dose of Nnavitoclax, the difference in the rate of change in tumor size (from pre-treatment to the time of sacrifice) with ABT263 TD1 and the rate of change in tumor size with TD1 alone will be assessed either using a two-sample t-test or a Wilcoxon rank sum test. The difference in the rate of change in tumor size with the combination and that vehicle will be examined. The dose level of Nnavitoclax +D1 that differs most from TD1 alone will be carried forward into the next experiment.

- The third experiment will test whether adding a HER2 inhibitor add to combination's ability to inhibit tumorigenicity. Mice will be randomized to each of the following treatment groups when their tumors have reached a size of 100 mm3:TD1 + Nnavitoclax dose from experiment 2 (Nnavitoclax D1) and TD1 + Nnavitoclax D1+ Trz (15mg/kg/week/i.p) TD1+ Nnavitoclax (300 nM). Mice will be sacred when the tumor reaches a size of about 2.0 cm2 or appears in distress. The difference in the rate of change in tumor size with the combination of TD1 + Nnavitoclax D1 and the rate of change in tumor size with TD1 + Navitoclax D1+trastuzumab as well as difference in the rate of change in tumor size with the triplet and the rate of change in tumor size with the vehicle will be assessed either using ANOVA.

- For each of the 3 experimental studies we will use 10 mice per treatment group, a two-sided α=0.01 t-test of the difference in two means will have a 90% change of detecting a 2 STD difference in treatment means. To elucidate the correlation between FASN expression, treatment outcome and cell death, tumors will be stained for *in vivo* apoptosis using ApopTag Kit. Ki67 expression will be assessed to determine proliferation. Additional markers could be stained as we see them fit during the study. Tumor measurements and metastasis will be followed by the ***Fluor Vivo system*** for fluorescent signal (cells labeled with GFP) and Xenogen system for luminescent signals (cells labeled with Luciferase).

3.2: What accomplished under these goals?

- **Major Task 3 (original proposal Major Task 10):** Preclinical assessment of the FASN inhibitor TVB-3166 in combination with ABT263.
    - Preclinical Studies BEAUTY:

REPRESENTATIVE RESULTS (From the new SOW)

- **Major Task 3:** Preclinical assessment of the FASN inhibitor TVB-3166 or the news compound TVB-3664 (Investigational compounds for animal use matching the clinical TVB-2640 oral treatment) in combination with ABT263.

A) The initial studies were completed using BT-474 breast cancer Xenograft tumors treated with inhibitor of FASNi (TVB3664) in combination with either: **Venetoclax a selective Bcl2 inhibitor (ABT-199) or Nnavitoclax is a Bcl2 and Bclxl inhibitor (ABT-263).**

A clinical grade FASNi enhances sensitivity to navitoclax and venetoclax *in vivo*: We finally sought to determine the efficacy of combining navitoclax/ABT-263 or venetoclax/ABT-199 with TVB-3664 against BT-474 human breast cancer xenografts in nude mice. BH3 mimetics and TVB-3664 were administered by oral gavage to mimic human oral drug administration. Both navitoclax and venetoclax failed to elicit any tumor growth delay of BT-474 xenograft tumors; notably, single agent TVB-3664 was notably efficacious in

producing a tumor response (44% tumor growth inhibition) (Fig. 1). The completely lack of anti-tumor efficacy of navitoclax and venetoclax as single agents, were fully circumvented when FASN activity was pharmacologically targeted in BT-474 tumor xenografts; thus, when administered in combination with the FASNi TVB-3664, navitoclax and venetoclax caused strong tumor growth inhibition (80% and 78%, respectively; Fig. 1). Combination therapy were well-tolerated, with mice maintaining normal body weight.

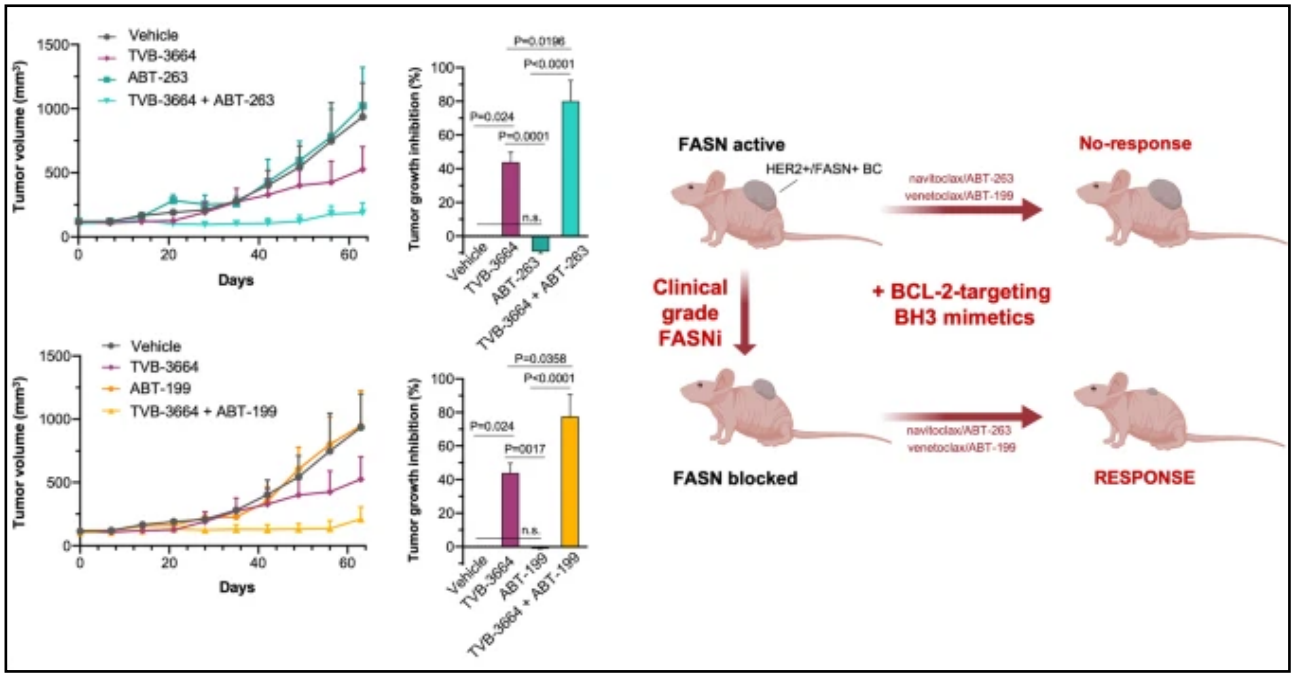


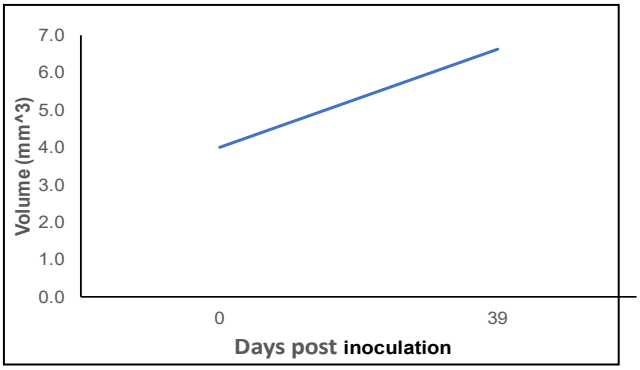
Figure 1: FASN inhibition sensitizes human breast tumor xenografts to BCL-2-targeting BH3 mimetics *Left*. Growth of BT-474 xenograft tumors in athymic female mice treated with BH3 mimetics navitoclax/ABT-263 (*top*) and venetoclax/ABT-199 (*bottom*) in the absence or presence of the FASNi TVB-3664. The maximum length for each treatment was 63 days. Results are shwn as the mean tumor volume ± S.D. ($n = 10$ mice/experimental group). Tumor growth inhibition (TGI) was calculated as the percentage of tumor growth, relative to tumor size at the start of treatment, in drug-treated groups compared to vehicles-treated group. *Right*. *In vivo* findings from the HER2 + /FASN-overexpressing breast cancer model BT-474 uncovers a novel FASN-dependent mitochondrial priming that links *de novo* FA biosynthesis to the intrinsic apoptotic threshold in breast cancer cells. The discovery that FASN-inhibited cancer cells exist in an apoptosis-prone state highly sensitive to BCL-2-targeting BH3 mimetics might warrant clinical exploration in patients with HER2 + /FASN-addicted breast carcinomas (see the discussion section). FASN inhibition increases mitochondrial priming and enhances breast cancer cell sensitivity to BCL2-targeting BH3 mimetics: a working model.

B) Next studies were completed using <u>HER2 positive PXD tumors</u> derived from the tumor repository of the BEAUTY clinical trial.

Mayo Clinic BEAUTY consortium provided our laboratory, initially, with two humans HER2+ breast cancer PDX tumors (namely BJ-11 and BJ-07). The tumors where immediately implanted, for expansion, in the immunodeficient mouse model NSG mice (SCID). The studies were performed under the consortium IACUC.

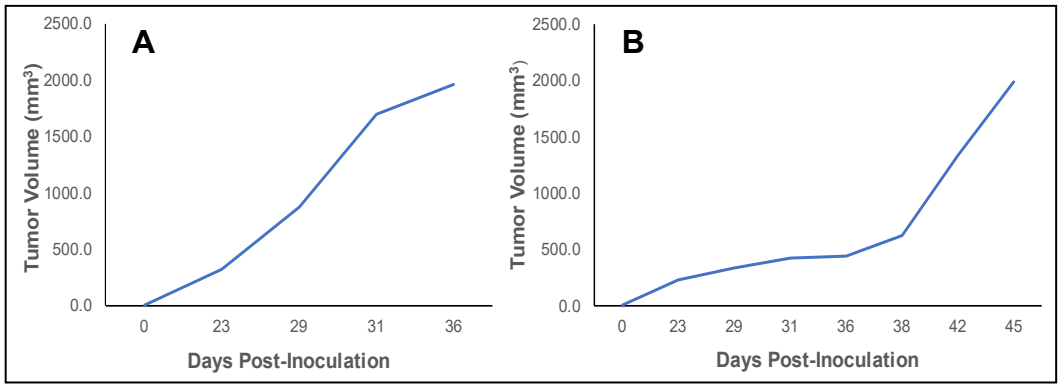
<u>*B1: The BJ07 tumors were implanted (4mm^3).*</u> Tumors were monitored and measured by calipers by measuring length and width to determine tumor volume (volume=0.5*(length x width). Tumor on 11/14/22 the tumor was a bump and measured approximately 6.6mm (measuring that small there is reasonable error) See Figure 2.

Figure 2: Development of HER2/PDX/BJ07-Tumor Growth Curve. BJ07 tumor obtained from Mayo Clinic BEAUTY Consortium. BJ07 tumor slices (4mm^3) were implanted into the 4th mammary fat pad and allowed grow. Mice were monitored several times a week to ensure tumor growth and mouse wellness. Tumors volumes were determined by measuring length and width of tumor and calculating the tumor volume (V=1/2(length x width^2). was implanted into one NSG mouse (Day 0) and was monitored for tumor growth. Calipers were used to measure the tumor length and width. Tumor volume was determined by the equation V=1/2(length x width^2).



B2: The BJ11 tumors were implanted (4mm^3). Tumors were monitored and measured by calipers by measuring length and width to determine tumor volume (volume=0.5*(length x width^2). Upon tumor reaching about 1.8 mm^3, mice were sacrificed, and tumors were diced-up and implanted for the expansion and the additional studies. See Figure 3.

Figure 3: Development of HER2/PDX/BJ11-Tumor Growth Curve: Two NSG mice (Mouse A and B) were implanted with BJ11 slices (4mm^3) into the 4th mammary fat pad and allowed to grow. Mice were monitored several times a week to ensure tumor growth and mouse wellness. Tumors volumes were determined by measuring length and width of tumor and calculating the tumor volume (V=1/2(length x width^2). Upon tumors reached up to no more than 1.8 mm^3. At that point, mice were sacrificed, tumors were removed and 4mm^3 slices were re-implanted into 3-4 old week female NSG mice for propagation of tumors in additional 15 mice to further generate tumor bearing mice to begin the experiments detailed in Task 3.



Experimental Design: Due to the constrain in the tumor development in the re-implantation of the tumors, we had to begin the experiment with the BJ11 PDX and we are still awaiting the additional PDX. In addition, the tumor take it has been quite variable, therefore, the experimental design was modified as follows:

Group	Treatment	Number of Mice
Group 1	Vehicle Control	6
Group 2	2mg/kg TVB3664	6
Group 3	4mg/kg TVB3664	6
Group 4	25mg/kg ABT263	6
Group 5	50mg/kg ABT263	6
Group 6	2mg/kg TVB + 25mg/kg ABT263	6
Group 7	2mg/kg TVB + 25mg/kg ABT263	6
Group 8	4mg/kg TVB + 50mg/kg ABT263	6
Group 9	4mg/kg TVB + 50mg/kg ABT263	6

The animal study will be completed in the next month or two.

What opportunities for training and professional development has the project provided?
Nothing to report.

How were the results disseminated to communities of interest?
Nothing to report.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**

Nothing to report at this time.

- **What was the impact on other disciplines?**

Nothing to report at this time.

- **What was the impact on technology transfer?**

Nothing to report at this time.

- **What was the impact on society beyond science and technology?**

Nothing to report at this time.

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**

Nothing to report at this time.

- **Actual or anticipated problems or delays and actions/plans to resolve them.**

The specimens for the correlative studies are currently being collected form all three Mayo sites and will be conducted in the next month or two.

- **Changes that had a significant impact on expenditures**

Nothing to report at this time.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.**
  - **Significant changes in use or care of human subjects**

  Nothing to report.
  - **Significant changes in use or care of vertebrate animals**

  Nothing to report.
  - **Significant changes in use of biohazards and/or select agents.**

  Nothing to report.

6. PRODUCTS:

Publications, conference papers, and presentations

Journal publications

1. Elisabet Cuyàs, Salvador Fernández-Arroyo, Sara Verdura, **Ruth Lupu**, Jorge Joven, Javier A Menendez Metabolomic And Mitochondrial Fingerprinting Of The Epithelial-To-Mesenchymal Transition (EMT) In Non-Tumorigenic And Tumorigenic Human Breast Cells. Cancers (Basel) 2022 Dec 16;14(24):6214

2. Javier A Menendez and **Ruth Lupu**, Fatty acid synthase: a druggable driver of breast cancer brain metastasis, Expert Opin Ther Targets. 2022 May;26(5):427-444.

Books or other non-periodical, one-time publications

Nothing to report.

Other publications, conference papers, and presentations

Nothing to report.

Website(s) or other Internet site(s)

Nothing to report.

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

Nothing to report.

6. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

No Change

What other organizations were involved as partners?

We continue collaboration with 3V-Biosciences, Inc. (Renamed SAGIMET Inc.)

Organization Name: SAGIMET, Inc.
Location of Organization: 3715 Haven Ave. Suite 220, Menlo Park, CA 94025

Partner's contribution to the project: Sagimet is providing the investigational agent, TVB-3166, TVB3446, TVB-2640. A pathologist will oversee serum FASN and tissue pAKT and pS6 correlative studies

Financial support: Financial support from 3V Biosciences is not provided to Mayo Clinic, Dr. Haddad, or the clinical trial participants.

In-kind support:
Not applicable
Facilities:
Not applicable

Collaboration: Scientists from Sagimet (3V Biosciences) will:
- Review study safety data and assist with safety monitoring.
- Participate in data interpretation, as appropriate.

Personnel exchanges:
Not applicable

Name:	*Ruth Lupu*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	0000-0001-8226-3581
Nearest person month worked:	1.8
Contribution to Project:	Authored the Translational research and contributed all the preliminary data for the research proposal except the clinical trial data. Led training and logistics review for the laboratory study personnel; facilitated contract completion with 3V Biosciences; active oversight the research and the collaborative studies
Funding Support:	

Other:
Not applicable

Pending Proposals:

Department of Defense BCRP Breakthrough Award - Funding Level 2 - Partnering PI Option (Javier Menendez)
BC210816P1: Title: "Unknown yet"
Funding Period: 04-30-20123– 03-29-2027

RO1-NIH/NCI- To be submitted by July 5th, 2023.
Title: Co-Targeting Fatty Acid Synthase (FASN) and Sphingosine Phosphate Kinase 1 (SphK1)/Sphingosine 1-Phosphate (S1P) Axis in Hormone Receptor Positive (HR+)/Hormone-Resistant Metastatic Breast Cancer

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS

Dr. Ruth Lupu, PhD. Principal Investigator (PI)

Dr. Haddad is the Partnering PI.

QUAD CHARTS:

Nothing to report.

APPENDICES:

No Appendices