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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)
Taxol™
Cancer metabolism
Drug resistance
Clinical Trial
Biomarkers

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: ***To 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 8: Mechanism of apoptotic synergy between FASN inhibition and PXL**
  Milestone in progress: Study mostly completed and reported

- **Major Task 9: Linking FASN inhibition to increased ROS production**
  Milestone in progress: Completed

- **Major Task 10: Preclinical assessment of the FASN inhibitor TVB-3166 (the form of TVB-2640 for animal use) in combination with ABT263**
  Milestone in progress: Study is partially completed.

3.2: What accomplished under these goals?

- **Major Task 8: Mechanism of apoptotic synergy between FASN inhibition and PXL**

Subtask 1:

- Determine which of the BH3-only proteins is regulated by modulation of PXL and FASN
- Overexpress BH3-only proteins determine whether the synergistic effect is reversed
- Downregulate the gene of interest causes sensitization to TVB and PXL.

- **Major Task 9: Linking FASN inhibition to increased ROS production**

Subtask 1: Tumor biospecimens stained, scored and interpreted

- Determine the effect of FASN inhibition on lipid composition of the mitochondrial
- Membrane (In different models of FASN expression in breast cancer)
- Determine the effect of FASN inhibition on oxidative stress and redox unbalance (In different models of FASN expression in breast cancer)

REPRESENTATIVE RESULTS:

➢ **Major Task 8: Mechanism of apoptotic synergy between FASN inhibition and PXL**

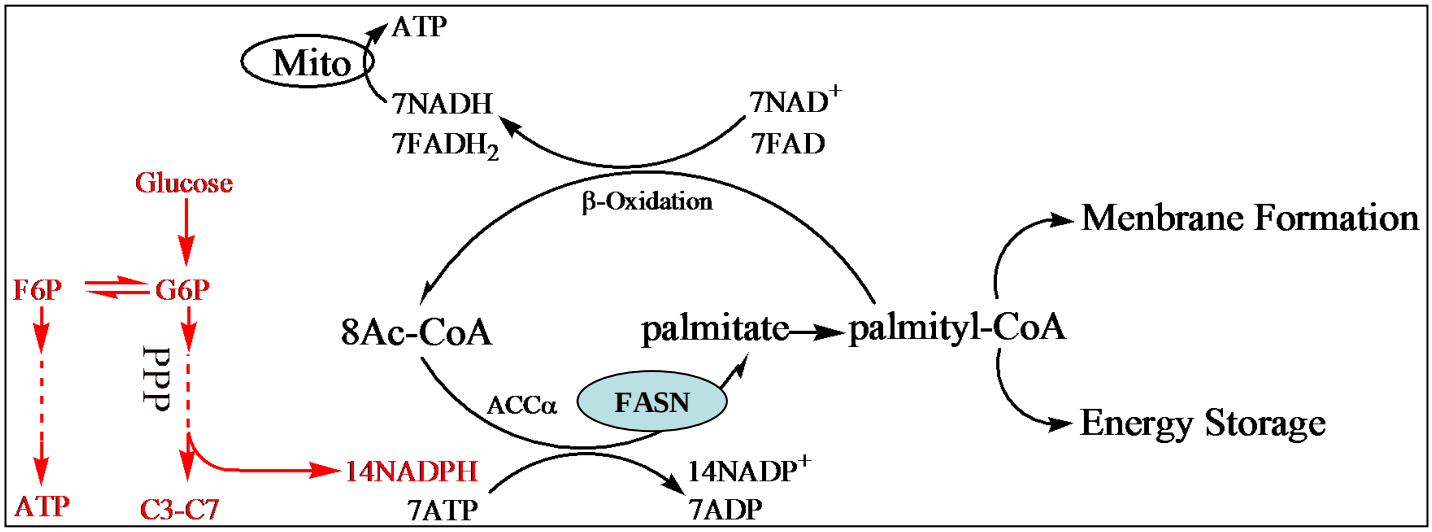
Subtask 1:

- Determine which of the BH3-only proteins is regulated by modulation of PXL and FASN
- Overexpress BH3-only proteins determine whether the synergistic effect is reversed
- Downregulate the gene of interest causes sensitization to TVB and PXL.

Milestone Achieved: Understanding how FASN inhibition promotes taxane sensitivity. Report final studies and publish results

A highly complex rewiring of metabolic pathways orchestrated to meet or even exceed the increased metabolic demands of cancer cells [1-4]. Elevated *de novo* fatty acid biogenesis driven by the overexpression and hyperactivation of several lipogenic enzymes is one of the most common cancer-associated metabolic traits that provide proliferative and survival advantages to tumors. Fatty acid synthase (FASN) is a key enzyme in the endogenous lipogenesis pathway that primarily catalyzes the synthesis of the long-chain saturated fatty acid palmitate from acetyl-CoA and malonyl-CoA, using NADPH as a reducing agent. FASN activation is an early and near universal hallmark of most human carcinomas and their precursor lesions, and is enhanced in a stage-dependent manner that associates with worsened patient survival and therapeutic resistance in several cancer types. Cancer cells utilize FASN endogenously-produced free fatty acids for phospholipid synthesis of new membranes, for pro-survival signaling molecules (e.g., sphingolipids) and for obtaining energy via β-oxidation (**Fig. 1**).

Figure 1: Function of overexpressed FASN in proliferating cells



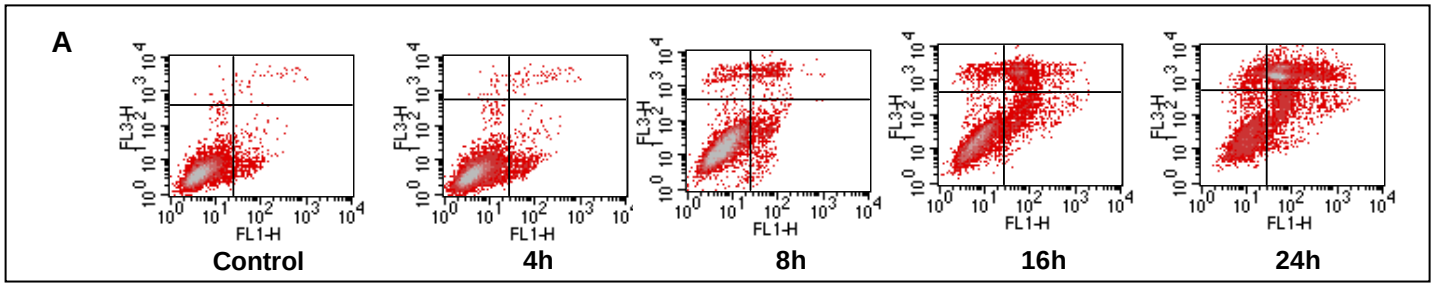
FASN provides fatty acids for membrane formation. The cycle of fatty acid synthesis and oxidation transforms NADPH to ATP so that PPP could normally provide different sugar for metabolism. The cycle of fatty acid synthesis and oxidation transforms NADPH to ATP so that PPP could normally provide different sugar for metabolism.

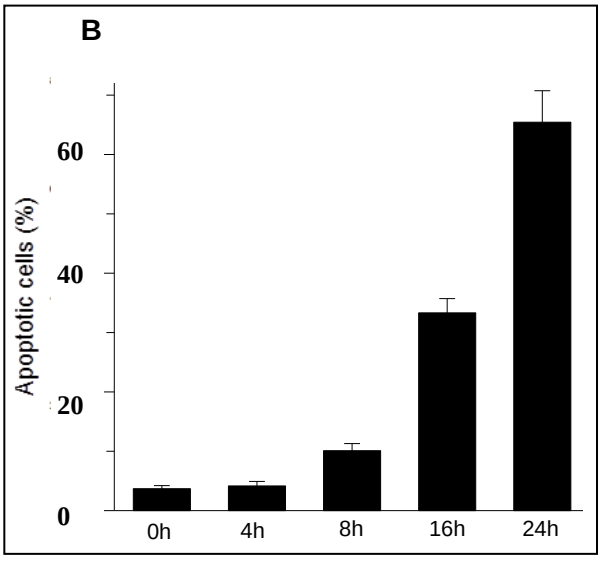
Interest in FASN as a target for therapeutic intervention stemmed from findings more a decade ago that tumor cells addicted to FASN-driven lipid signaling show significantly reduced growth and viability upon FASN inhibition. Since then, however, we have been unable to resolve the apparent discrepancy between the basic

science-discovery *bench* aspects of FASN blockade and the awaited *bedside* effects of clinical-grade FASN inhibitors (FASNis).

The extent of FASN inhibition-induced apoptotic cell death relates to treatment time related manner. Apoptosis was monitored by flow cytometry after staining with annexin V, which binds phosphatidylserine that is exposed during apoptosis. FASN inhibition significantly and dose-dependently increased the number of annexin V-positive BT-474 cells **Fig 2A** flow cytometry results**,** and **Fig 2B** quantification, relative to vehicle-treated control cells (not shown).

Figure 2A-B: Action of FASN inhibition in BT474 cells induces



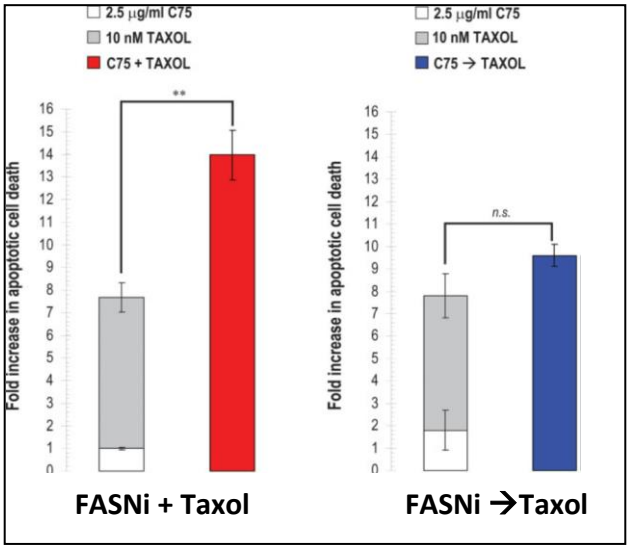


These results show that the extent of apoptosis in response to FASN inhibition reflects the baseline level of FASN expression, suggesting an augmented dependency of cancer cell survival on FASN activity.

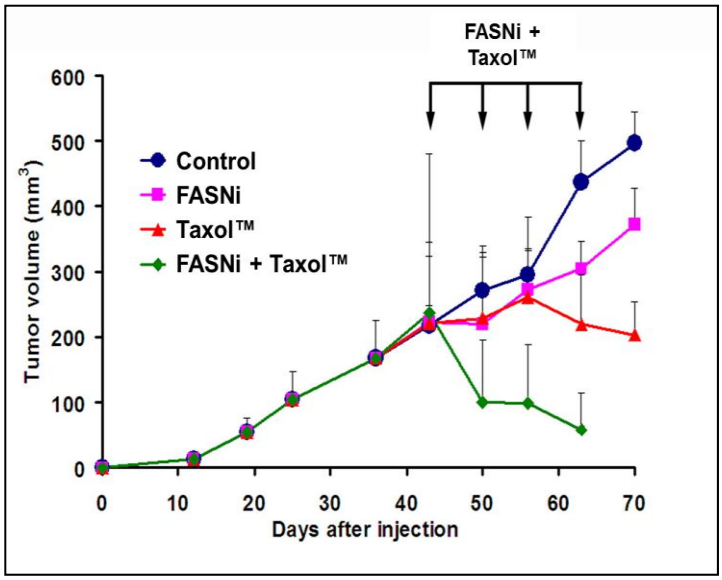
The relationship between FASN and chemotherapy-induced cell damage has not been well studied. We examined the ability of FASNi to modulate the cytotoxic activity of the microtubule-interfering agent Taxol™ (paclitaxel) in breast cancer cells. When the combination of FASNi with Taxol™ in either concurrent (FASNi + Taxol™ 24 hr) or sequential (FASNi 24 hr → Taxol™ 24 hr) schedules were tested for synergism, addition or antagonism using the isobologram and the median-effect plot analyses, co-exposure of FASNi and Taxol™ mostly demonstrated synergistic effects, whereas sequential exposure to FASNi followed by Taxol™ mainly showed additive or antagonistic interactions. We next evaluated the effects of FASNi on Taxol™-induced apoptosis as well as Taxol™-activated cell death and cell survival-signaling pathways in this breast cancer cell model. Co-exposure to FASNi and Taxol™ induced a remarkable nuclear accumulation of activated p38 mitogen-activated protein kinase (p38 MAPK), which was accompanied by a synergistic nuclear accumulation of the p53 tumor-suppressor protein that was phosphorylated at Ser46, a p38 MAPK-regulated pro-apoptotic modification of p53.

Our findings establish for the first time that FASNi augments the cytotoxicity of anti-mitotic drug Taxol™ against breast cancer cells and that this chemosensitizing effect is schedule-dependent. We suggest that the alternate activation of both the pro-apoptotic p38 MAPK-p53 signaling and the cytoprotective MEK1/2 → ERK1/2 cascade, as well as the inactivation of the anti-apoptotic AKT activity may explain, at least in part, the sequence-dependent enhancement of Taxol™-induced cytotoxicity and apoptosis that follows inhibition of FASN activity in breast cancer cells.

Pharmacological inhibition of FASN activity synergistically enhances Taxol™-induced apoptosis in a schedule-dependent manner: We next evaluated the possibility that the synergistic decrease in cell viability of breast cancer cells co-treated with the FASNi and Taxol™ would represent a cerulenin-promoted enhancement of Taxol™-induced programmed cell death (apoptosis). First, MCF-7 cells were co-exposed to FASNi and Taxol™ for 24 hr, and apoptotic cell death was measured by the Cell Death Detection ELISA, which is based on quantitative enzyme-immunoassay-principle using mouse monoclonal antibodies directed against DNA and histones that allows the specific determination of mono- and oligo nucleosomes that are released into the cytoplasm of cells dying from apoptosis. In this treatment, we used the lowest clinically relevant concentration of Taxol™ that blocks normal cell cycle progression at the G_2-M phase of the cell cycle (10 nM). With this protocol, Taxol™ by itself induced a 7-fold increase in basal apoptosis (*e.g.*, *vs.* untreated cells), whereas administration of a sub-optimal concentration of FASNi alone exerted almost negligible effects on apoptotic cell death of MCF-7 cells (**Fig. 3, *left panel***). More importantly, the inhibition of FASNi together with Taxol™ resulted in an enhancement of apoptosis that was significantly higher than the additive value of the 2 drugs alone. Thus, FASNi and Taxol™ combined caused 2 times more apoptotic cell death than Taxol™ alone, and 14 times more apoptotic cell death than FASNi alone. A completely different picture emerged when MCF-7 cells were pre-treated with FASNi for 24 hr prior Taxol™ exposure. Thus, sequential administration of FASNi followed by Taxol™ exerted little effects on Taxol™-mediated apoptosis (**Fig.3, *right panel***). Consistent with previously observed sequence-dependent synergism using the isobologram and Chou and Talalay cytotoxic analyses, these findings suggest that co-exposure of MCF-7 breast cancer cells to FASNi and Taxol™ is necessary for maximal augmentation of Taxol™-induced apoptotic cell death, whereas sequential administration FASNi by Taxol™ did not increase apoptosis relative to cells exposed to Taxol™ alone. FASNi **-induced inhibition of FASN activity synergistically enhances Taxol™-induced apoptotic cell death in a schedule-dependent manner. MCF-7 cells were left untreated or treated with 2.5 µg/ml FASNi, 10 nM Taxol™, or a combination of FASNi + Taxol™ using different schedules of administration.**



These data demonstrated that FASN modulates the chemotherapy response in vitro suggesting that FASN can be a molecular target to enhance the efficacy of taxane-based chemotherapy in vivo.



FASN inhibition sensitizes breast cancer tumors to Taxol™ treatment: We demonstrated that blockage of FASN sensitizes breast tumors to Taxol™. We found that FASNi plus Taxol™ inhibited the tumor growth 9 fold and reduced the tumor size 4 fold compared to the control mice (no treatment). In comparison, FASNi alone reduced the tumor growth 1.5 fold and Taxol™ 2.5 fold compared to the control mice **Fig 4**.

Orthotopic tumor growth of BT474 cells in athymic nude mice: BT474 cells ($4x10^6$) were injected into the mammary fat pad of athymic nude-Foxn1nu mice (3-4 weeks old). Once the tumors were ≤150 mm^3 mice

were randomized and treated once a week with intraperitoneal injections of FASNi (2mg/kg), Taxol™, (10mg/kg) or the combination of both. Tumor growth was monitored by measurement once a week. Tumor volumes were calculated by three-dimensional measurements using the following formula: tumor volume (mm^3) = length x width x height/2. Graph showing the means ± SE of tumor growth of each group (n=10) for up to 70 days.

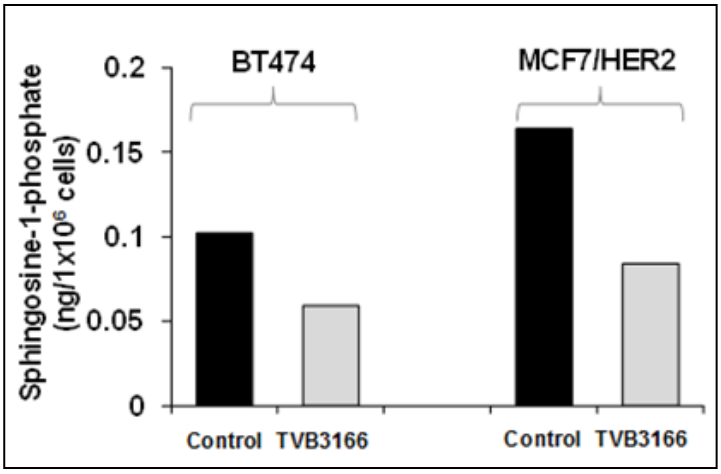
The results show for the first time that blockage of FASNi sensitizes breast cancer tumors to Taxol™ treatment in vivo confirming the clinical value of FASN as a therapeutic target.

- **Major Task 9: Linking FASN inhibition to increased ROS production**

Subtask 1: Tumor biospecimens stained, scored and interpreted

- Determine the effect of FASN inhibition on lipid composition of the mitochondrial
- Membrane (In different models of FASN expression in breast cancer)
- Determine the effect of FASN inhibition on oxidative stress and redox unbalance (In different models of FASN expression in breast cancer)

Determine the effect of FASN inhibition on lipid composition of the mitochondrial: we assess the effect of FASN using FASNi on the lipid profile of breast cancer cells. Isolated mitochondrial membranes from cells pre- and post-treatment were tested. We expect that FASNi will alter the lipid composition of the inner mitochondrial membranes, as opposed to the untreated control membranes. One such lipid was remarkably downregulated in breast cancer cells expressing high levels of FASN. It appears that FASN selectively regulates the level of Sphingosine 1-phosphate (S1P), suggesting that this bioactive lipid might be a potential mediator of FASN induced breast carcinoma. **Fig 5** shows two breast cancer cells cell, in both cases FASNi block significantly the synthesis of S1P. BT-474 and MCF-7/HER2-18 cells express high levels of FASN, HER2 and ER. No change in S1P levels was observed, when MCF-7/neo cells were tested. MCF-7/neo cells are ER+ but do not express detectable levels of either HER2 or FASN.



FASN is link to sphingolipid metabolism: The levels of S1P were measured in BC cells after FASN knockdown cells: To assess whether there is link between FASN expression and sphingolipid metabolism, FASN expression was knockdown using a FASNsh in BT-474, MCF-7/neo and MCF-7/HER2-18 (data not shown)

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

What do you plan to do during the next reporting period to accomplish the goals?

- **Major Task 8: Mechanism of apoptotic synergy between FASN inhibition and PXL**
  - Downregulate the gene of interest causes sensitization to TVB and PXL. (continue)
- **Major Task 9: Linking FASN inhibition to increased ROS production**
  - Determine the effect of FASN inhibition on lipid composition of the mitochondrial (continue)
  - Membrane (In different models of FASN expression in breast cancer) (continue)
- **Major Task 10: Preclinical assessment of the FASN inhibitor TVB-3166 (the form of TVB-2640 for animal use) in combination with ABT263**
  - The recently completed pre-clinical trial (in vitro studies) (continue)
  - Histopathological assessment of Tumor derived from the *in vivo* studies

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**

Tasks related to tissue and serum specimens will be delayed due to delay in the clinical trial (Explained in Dr. Tufia Haddad's progress report)

- **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

5. **PRODUCTS:**

Publications, conference papers, and presentations

Abstract submitted and ACCEPTED to the Annual conference of the American Association for Cancer Research (April 2019) Fatty Acid Synthase: A Therapeutic Target: Travis Van der Steen, George Kemble, and Ruth Lupu

Journal publications

Manuscripts accepted to be published

1) Papadimitropoulou A, Vellon L, Atlas E, Steen TV, Cuyàs E, Verdura S, Espinoza I, Menendez JA, **Lupu R.** Heregulin Drives Endocrine Resistance by Altering IL-8 Expression in ER-Positive Breast Cancer.
2) Menendez JA, Mehmi I, Papadimitropoulou A, Vander Steen T, Cuyàs E, Verdura S, Espinoza I, Vellon L, Atlas E, **Lupu R.**

Manuscripts submitted for Publication

1) Espinoza I, Vander Steen T, Schroeder B, Cuyàs E, Kurapaty Venkatapoorna CM, X. Wei Meng, Schneider PA, Regan K, Flatten KS, Verdura S, Kaufmann SH, Menendez, JA, **Lupu R.** Fatty acid synthase regulates the mitochondrial primed-for-death state in breast cancer cells. Submitted to: Cell Dead and Differentiation.

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

Technologies or techniques

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?

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:	

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: 3V Biosciences is providing the investigational agent, TVB-2640, and the company 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 participant's

In-kind support:

Facilities:

Not applicable

Collaboration: Scientists from 3V Biosciences will

- Review study safety data and assist with safety monitoring
- Participate in data interpretation, as appropriate

Personnel exchanges:

Not applicable

Other:

Not applicable

Pending

P50 CA102701: Mayo Clinic SPORE in Pancreatic Cancer

National Cancer Institute.

Title: “Targeting Fatty Acid Synthase: A Mechanism-Guided Approach to Target Pancreatic Adenocarcinoma and the Tumor Microenvironment”

Funding Period: 09/1/2020 – 08/ 31/2025

Overall PI: Billadeau D.

Role: PI- Project # 2

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