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TITLE: Targeting GD3 Synthase (ST8SIA1) in GD2+ Breast Cancer Stem-Like Cells to Prevent Tumor Growth and Metastases in Triple-Negative Breast Cancer

PRINCIPAL INVESTIGATOR: Venkata Lokesh Battula, Ph.D.

CONTRACTING ORGANIZATION: University of Texas MD Anderson Cancer Center

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

In this study, we aim to investigate the role of GD3 synthase (ST8SIA1 or GD3S) in triple-negative breast cancer (TNBC) progression, especially in the regulation of cancer stem cell function. We have reported that Ganglioside GD2 and GD3S are overexpressed in TNBC cancer stem-like cells, and inhibition of GD3S expression or its activity hampers TNBC tumor growth and metastasis. During the first year of this study, we successfully obtained approvals for IRB, IBC, and IACUC (ACURO) protocols and recruited experienced postdoctoral fellows to work on this project. We have optimized different molecular methods for measuring GD2 and GD3S expression in primary tumor samples as well as stratifying TNBC patients into different subtypes based on their gene expression patterns. To target GD3S in cancer stem cells, we identified two FDA approved drugs using protein homology modeling and docking strategy. In addition, we identified specific mutations in the TP53 gene that regulate GD3S expression in breast cancer. We will validate these findings using animal models during the next phase of the study.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	20	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

   In this proposal, we aim to identify drivers of breast cancer growth by targeting GD3 synthase (ST8SIA1) to inhibit BCSCs' functions, including tumor initiation, growth, and metastasis, in triple-negative breast cancers (TNBCs). In addition, we will identify the mechanism of GD3 synthase-regulated tumor initiation and metastasis of BCSCs in TNBCs. Our preliminary data confirms that GD2 selectively identifies BCSCs in TNBCs and that the enzyme GD3 synthase regulates GD2 biosynthesis. Analysis of TCGA data demonstrated that GD3 synthase expression is higher in basal-like TNBC tumors than in other molecular subtypes of breast cancer. Moreover, we found that GD3 synthase expression is significantly upregulated in TNBC tumor samples with p53 mutations. We also found that knockdown of GD3 synthase expression in TNBC cell lines inhibits tumor growth and metastasis *in vivo*, whereas GD3 synthase overexpression induces epithelial-to-mesenchymal transition, leading to metastasis of TNBC cells. The key mechanism we identified is GD3 synthase mediated regulation of FAK/AKT/mTOR signaling pathway in $GD2^+$ BCSCs. In addition, we found that inhibition of *GD3 synthase* expression dramatically inhibited mitochondrial respiration and increased ceramide levels in TNBC cells. Here we propose to determine the effect of novel GD3 synthase inhibitors on TNBC growth and metastasis in patient-derived xenograft models and identify the mechanism of GD3 synthase-mediated regulation of tumor TNBC growth and metastasis.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

   BCSCs, TNBCs, GD2, GD3 synthase (ST8SIA1), GD2 synthase (B4GALNT1), GD3S inhibitors, virtual ligand screening, p53, mutant p53, hotspot mutations,

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

   What were the major goals of the project?
   List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

   <u>Major Goal of the Project:</u>
   The goal of this project is to target GD3 synthase in BCSCs using small-molecule inhibitors developed in our laboratory and determine the mechanism by which GD3 synthase regulates growth and metastasis of TNBC cells. We continue to work towards the 2 fundamental aims of this project:

Specific Aim 1: Determine the effect of novel GD3 synthase inhibitors on TNBC growth and metastasis in patient-derived xenograft models.	**Timeline**	**Percent completionStatus**
Major Task 1: Establish a strategy to effectively treat GD3 synthase-overexpressing TNBCs.		
Subtask 1: Identify FFPE TNBC blocks and prepare tissue microarrays for immunohistochemical staining for GD3 synthase.	Months 0-2	Completed
Subtask 2: Perform Immunohistochemical analysis of tissue microarrays to quantitate GD3 synthase expression.	Months 1-4	On-track
Subtask 3: Send the selected FFPE sections with GD3 synthase expression data to Insight Genetics to characterize TNBC subtypes in MD Anderson tumors.	Months 4-6	On-track
Local IRB/IACUC approval.	Months 0-3	Completed
Milestone achieved: Submit manuscript on GD3 synthase expression in different TNBC subtypes and its correlation with prognosis.	Months 6-9	Behind-Schedule
Major Task 2: Determine the optimal tolerated dose of GD3 synthase inhibitors and their effect on GD2 expression in TNBCs in vivo.		
Subtask 1: Synthesize clinical-grade GD3 synthase inhibitors for in vivo treatment. We will select the best GD3 synthase inhibitors with the highest capacity for inhibition of GD2 expression in vitro and chemically synthesize the compounds.	Months 4-6	Completed
Subtask 2: We will treat mice bearing TNBC PDX tumor models with different doses of GD3 synthase inhibitors and identify the lowest dose with the greatest effect on GD2 expression in vivo.	Months 6-8	Behind the Schedule
Subtask 3: Determine the off-target effects of GD3 synthase inhibitor by gene expression analysis of control and GD3 synthase inhibitor treated cells.	Months 8-10	Behind the Schedule
Major Task 3: Determine the effect of novel GD3 synthase inhibitors on tumor growth and metastasis in TNBC		
Subtask 1: Determine the effect of GD3 synthase inhibitors on TNBC growth. We will administer treatment to murine PDX models of TNBC with selected GD3 synthase inhibitors alone or in combination with chemotherapy and analyze tumor growth.	Months 9-13	Behind the Schedule
Subtask 2: Determine the effect of GD3 synthase inhibitors on TNBC growth. We will treat the PDX models with selected GD3 synthase inhibitors alone or in combination with chemotherapy and analyze	Months 11-15	Behind the Schedule

metastases.		
Subtask 3: Analyze primary tumors and organs, including the lungs, liver, brain, and bone, to investigate GD2 expression and metastases using histological methods.	Months 15-18	Behind the Schedule
Milestone achieved: Submit paper on the effect of GD3 synthase inhibitors on TNBC growth and metastasis.	Months 18-20	Behind the Schedule
Specific Aim 2: Identify the mechanism of GD3 synthase-mediated regulation of TNBC tumor growth and metastasis.		
Major Task 1: Determine the role of p53 mutations in regulation of BCSC function in vitro and in vivo.		
Subtask 1: Generate p53-KD TNBC cell lines carrying various p53 hotspot mutations.	Months 12-18	Completed
Subtask 2: Measure GD3 synthase and GD2 expression in p53-KD cells using RT-PCR and flow cytometry.	Months 18-20	Completed
Subtask 3: Test p53-KD cells for anchorage-independent growth to test determine their BCSC function.	Months 20-22	Completed
Subtask 4: In vivo tumor initiation and metastasis.	Months 22-26	On-track
Milestone achieved: Submit paper on role of mutated p53 in GD3 synthase expression and BCSC function.	Months 26-30	On-track
Major Task 2: Determine the effect of GD3 synthase inhibition on mitochondrial function.		
Subtask 1: Test the hypothesis that GD3 synthase inhibition causes mitochondrial dysfunction through upregulation of ceramide expression.	Months 28-30	Not started yet
Subtask 2: Determine the effect of GD3 synthase inhibition on mitophagy and autophagy.	Months 30-35	Not started yet
Milestone achieved: Submit paper on mechanism of TNBC tumor growth and metastasis inhibition by GD3 synthase inhibitors.	Months 34-36	Not started yet

Progress in accomplishing goals

Cephalothin and cefixime are the potent lead compounds identified inhibiting GD3 synthase.

Our preliminary data indicate that one of the compounds (ZINC02886919) from our virtual ligand screening, inhibits GD2 expression in TNBC cell lines and invitro tumorigenesis in a concentration-dependent manner. To identify and validate GD3 synthase inhibitors from FDA approved drugs based on these observations, we aimed to perform virtual screening of drugs for additional inhibitors to test their activity against TNBC tumor growth and metastases. We used the same homology model of GD3S to dock the FDA compound library to identify potential inhibitors of GD3 synthase. A dataset of 4,356 FDA approved compounds were retrieved from 5 different database libraries (BML-2800, BML-2832, Prestwick phytochemicals, Green Pharma, and Spectrum chemicals).

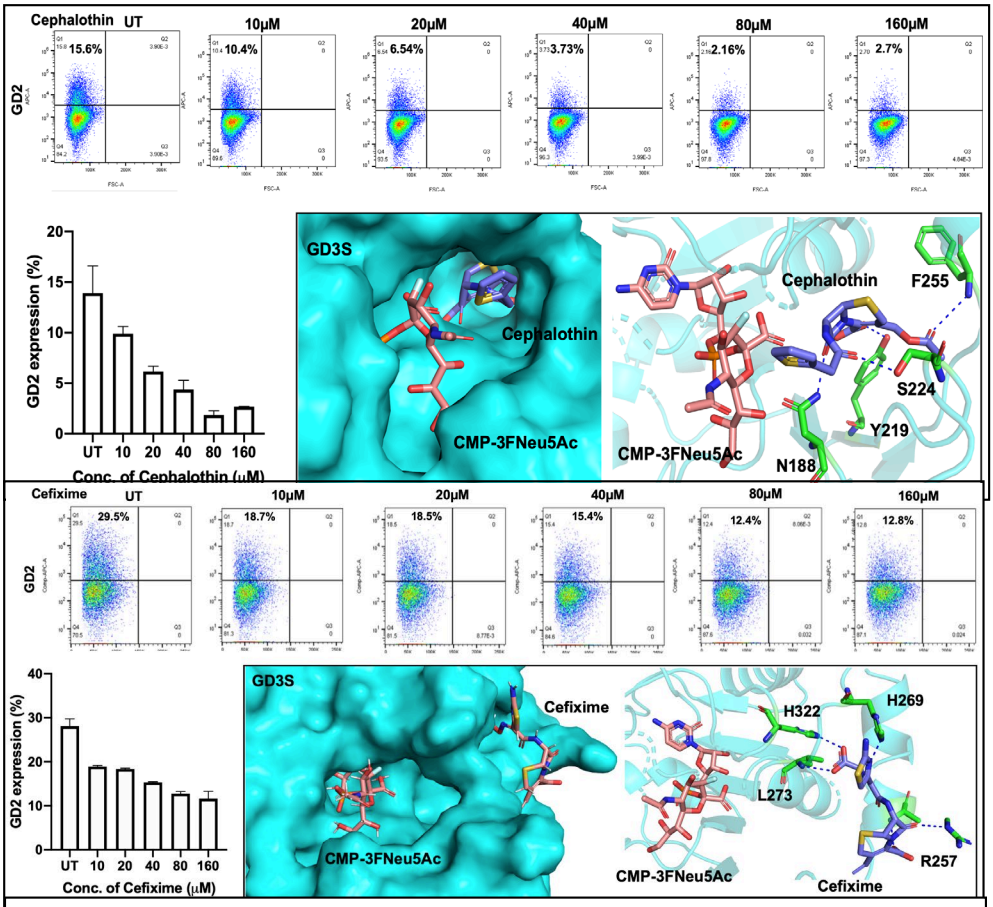

Figure 1. ST8SIA1 inhibitors target GD2+ cells and inhibit BCSC function. Chemical structure of, molecular formula for, and molecular weight of the potential ST8SIA1 inhibitor Cephalothin and Cefixime. SUM159 cells were treated with Cephalothin and Cefixime for 72h at different concentrations. Results of flow cytometric analysis of GD2 expression in the cells are shown.

The ligprep optimized ligands were docked into GD3 synthase protein model using the Virtual Screening Workflow (VSW) in Glide module available in Schrodinger modeling suite. Depending on glide XP score, the top 200 compounds were selected for further functional in vitro validation by performing a high throughput flow cytometry analysis. Out of all the tested compounds, cephalothin, cefixime, dioxybenzone, ibandronate sodium and tranilast showed an inhibitory pattern of GD2 expression in SUM159 cells. Among these identified compounds, cephalothin and cefixime treatment decreased the percentage of GD2 expression by 2- to 5-fold within 72 h in a concentration dependent manner (from 15.6% to 2.7% or 29.5% to 12.8% respectively). Interestingly, growth curve analysis showed no significant effect on the cell proliferation except at high concentration (160μM) notably in cephalothin treated SUM159 cells. This suggests that these compounds reduce

GD2 expression without exhibiting toxicity. In addition, cephalothin inhibited mammosphere formation for SUM159 cells by 10-fold while cefixime showed a moderate inhibition. This corroborates with the binding data analysis which showed a stronger affinity of -8.351 kcal/mol for cephalothin compared to -7.45 kcal/mol for cefixime. Moreover, cephalothin docked well into the substrate binding site while cefixime docked on the external cavity adjacent to the binding site. Collectively, these data indicate that the cephalothin and cefixime are the potent lead compounds similar to the previously identified ZINC02886919 inhibiting GD3 synthase. These compounds will be further validated by additional in vitro tumorigenesis assays and in vivo studies.

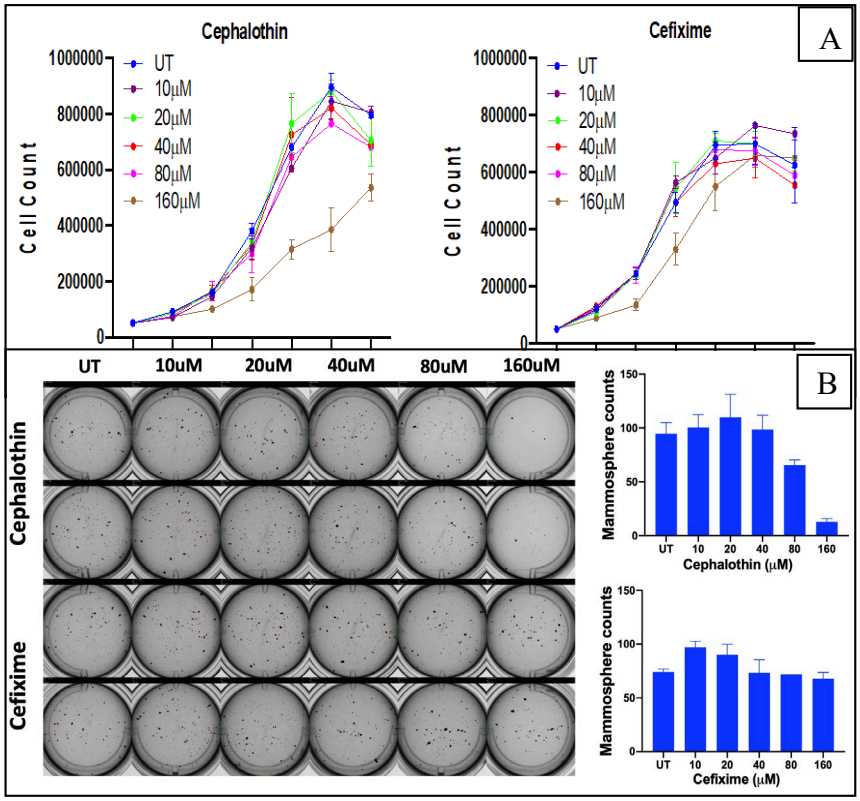

Figure 2. (A.) Growth curve analysis on SUM159 cells using predicted GD3S inhibitors. SUM159 cells ($5x10^4$/well) were cultured in 12-well cell culture dishes in duplicate and incubated for overnight attachment. Cells were treated with either cephalothin or cefixime at different concentrations. Following the treatment, total cells were counted from day 1 to day 6 using a Vi-CELL (Beckman Coulter) cell counter. **(B.) GD3S inhibitors reducing mammosphere formation.** SUM159 cells were seeded (5×10^3 per well) into low-adherent dishes containing mammocult medium with different concentrations (0, 10μM, 20μM, 40μM, 80μM and 160M) of cephalothin or cefixime. After 3 weeks, the mammospheres were stained with MTT reagent and counted by an automated colony counter.

Characterization of GD2 and GD3 synthase expression in primary TNBC tumors.

We have shown earlier that enzyme GD3S (GD3 synthase) regulates synthesis of ganglioside GD2 in TNBC. Furthermore, to substantiate our observation translationally, we want to characterize and clinically correlate the expression of GD2 and GD3 synthase in TNBC patient tissue samples. For this, we first endeavored to get full approval of our protocol by local IRB/ACURO/IACUC. After getting the approval we established the standardized protocol for IHC staining of FFPE TNBC sample for GD3 synthase. Furthermore, considering that GD2 is a ganglioside and we are receiving frozen TNBC tissue samples from institute's tissue bank, we developed IHC protocol for the frozen sections using anti-GD2 and anti-GD3 synthase antibody. We are employing this protocol in staining 200 TNBC frozen tissue specimens. To standardize the IHC protocol in frozen samples

with anti-GD2 and anti-GD3 synthase antibody we stably overexpressed GD3 synthase in MCF7 breast cancer cell line (low GD2 expressing) by lentiviral method. Flow-cytometry analysis confirmed that overexpression of GD3 synthase led to higher expression of GD2 (> 90%) in MCF7 cells compared to control (2.5%). As shown in figure 3, pellet of control MCF7 and GD3 synthase-overexpressing MCF7 cells were used to make frozen sections for the IHC analysis of GD2 and GD3 synthase in these cells. The standardized protocol with optimum concentration of both the antibodies is being used for IHC staining in 200 frozen TNBC tissue samples. We will clinically correlate the TNBC subtypes with the expression level of GD2 and GD3 synthase in these samples.

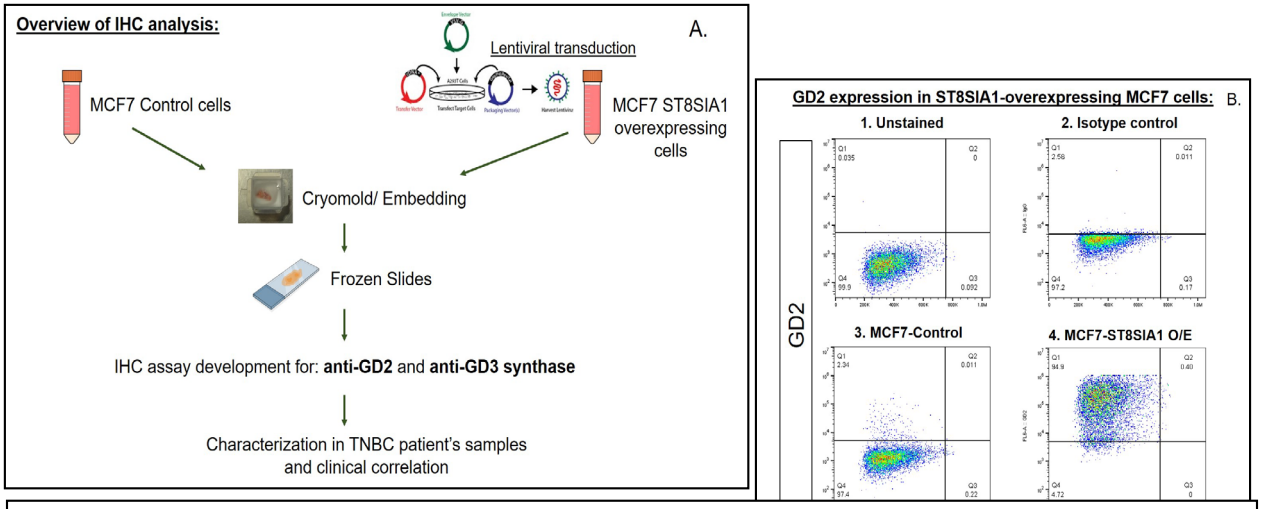

Figure 3: (A.) Schematic representation of assay-development for anti-GD2 and anti-ST8SIA1 immunohistochemistry (IHC) in frozen tissue samples of TNBC patients. Human breast cancer cell line MCF7 with low expression of GD2 was stably transduced by ST8SIA1-expressing vector to overexpress ST8SIA1. **(B.)** Flow-cytometry analysis confirmed the overexpression of ST8SIA1 by higher expression of GD2 compares to control cells. Control and ST8SIA1-overexpressing cells were used to generate frozen sections and assay development for GD2 and ST8SIA1 IHC-staining.

Characterization of p53 mutation status and expression levels in all available breast cancer cell lines

Using p53 cancer cell lines compendium, we characterized p53 mutation status in 24 available TNBC and non-TNBC cell lines. RPPA expression levels were derived from MD Anderson Cancer Cell Lines Project software. TNBC cell lines express several different p53 mutant forms. Seven TNBC cell lines (MDA-MB-468, Hs578T, HCC 70, HCC 38, BT549, HCC 1395, and SUM149) and 2 non-TNBC cell lines (SKBR3 and BT-474) have hotspot p53 mutations. On the other hand, 2 TNBC cell lines (DU4475 and HIM3) and 2 non-TNBC cell lines (MCF7 and ZR75-1) express wild type p53.

P53 expression levels vary greatly across all cell lines. In general, mutant p53 is stable and overexpressed, whereas low expression levels of wild type p53 are maintained through degradation by MDM2.

Characterization of GD2 and GD3 expression in breast cancer cell lines

Using Flow cytometry, we characterized expression of TNBC stem cell marker GD2 and its precursor ganglioside GD3. Expression levels of both markers vary greatly across cell lines. We

found that p53 mutant cell lines have a higher percentage of $GD2^+$ cells compared to wild type p53 cell lines (Median= 21 vs 4, $p < 0.02$). Additionally, cell lines with hotspot p53 mutations have a significantly higher percentage of $GD2^+$ cells compared to non-hotspot mutant cell lines (Median = 16.9 vs 0.69, $p<0.01$).GD2 and GD3 synthase are upregulated in basal-type TNBC cell lines and TCGA patient samples compared to other TNBC molecular subtypes.

GD3-synthase and GD2-synthase involved in regulating BCSCs function correlate with p53 mutation status

GD3 synthase expression (by qPCR) is significantly upregulated in TNBC cell lines with p53 hotspot mutations compared to cell lines with other p53 mutations (Median relative expression 0.0114 compared to 0.0003, p=0.005), suggesting that p53 hotspot mutations play an important role in the regulation of GD3s expression. Interestingly, GD2 expression is not always directly correlated with GD3s expression, suggesting that GD2s expression is also a key factor in the regulation of GD2 expression. In general, cell lines with high GD3s expression have a high percentage of $GD3^+$ cells. In cell lines with high GD2s expression (Hs578T and HCC38), GD3 acts as a precursor which is actively converted to GD2 (figure 4).

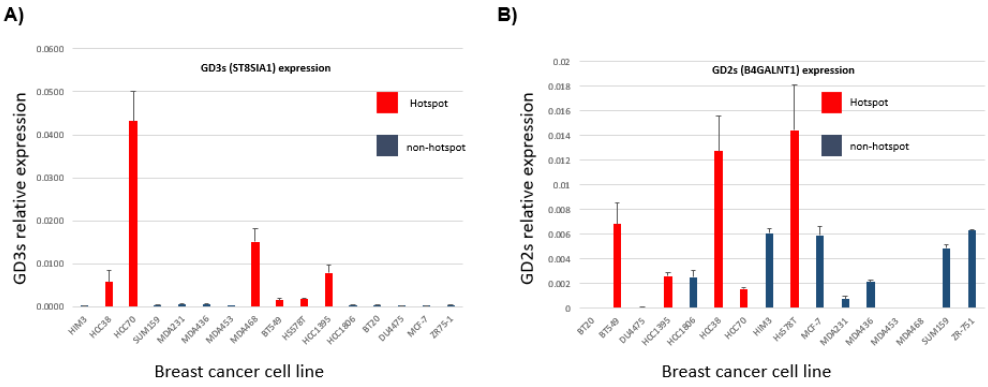

Figure 4: (A) 3 million cells were collected from each breast cancer cell line. RNA extraction was performed by ethanol precipitation. GD3 synthase expression was measured by RT-qPCR. **(B)** The same experiment was done as in **A** to measure GD2 synthase expression. Data are represented as relative fold increase in expression. Values were normalized to GAPDH. All samples were run in triplicates. Bar graphs are plotted as mean values ± standard error.

Wild type and mutant p53 demonstrate differential effect on BCSCs function

We demonstrated that 72 hours treatment of wild type p53 TNBC cell lines (DU4475 and HIM3) with a small-molecule MDM2 inhibitor (Nutlin3-a) inhibits degradation of wild type p53 and stabilizes its expression in a dose dependent manner (figure 5A).

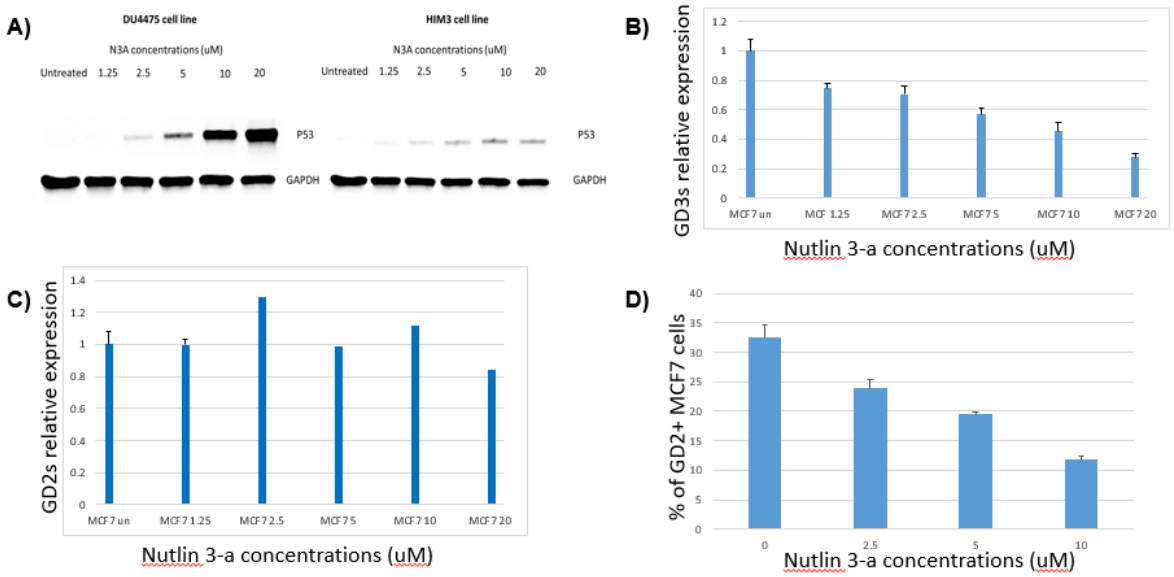

Figure 5: (A) Wild type p53 cell lines DU4475 and HIM3 were treated with the indicated concentrations of Nutlin3-a for 72 hours. Protein lysates were analyzed for p53 expression by western blotting. **(B)** MCF-7 cells were treated with the indicated concentrations of Nutlin-3a for 72 hours. RNA extraction was performed by ethanol precipitation, and GD3 synthase expression was measured by RT-qPCR relative to GAPDH. **(C)** The same experiment was performed as in **B** to measure GD2 synthase expression. **(D)** MCF-7 cells were treated with the indicated concentrations of Nutlin3-a for 72 hours. Cells were harvested and stained with anti-GD2 antibody. The percentage of GD2 expressing cells was measured by flow cytometry

Nutlin3-a treatment leads to a significant reduction of GD3s (but not GD2s) expression in a dose dependent manner in MCF7 cells, indicating that wild type p53 downregulates expression of GD3s (figure 5B, C). Downregulation of GD3s by stabilized wild type p53 leads to a resultant reduction of $GD2^+$ MCF-7 cells by flow cytometry (figure 5D)**.** To establish that hotspot p53 mutations promote TNBC stemness and tumorigenesis by upregulating GD3s expression, we performed lenti-viral mediated stable knockdown of mutant p53 in available TNBC cell lines with hotspot p53 mutations. We found that the majority of TNBC cell lines with hotspot p53 mutations did not survive following stable p53 knockdown, as compared to shRNA controls. This suggests that hotspot p53 mutant forms promote TNBC survival and cell proliferation through novel gain of function phenotypes. Out of 6 TNBC cell lines tested, only MDA-MB-468 cells survived mutant p53 knockdown (figure 6A). In MDA-MB-468 cells we validated p53 knockdown efficiency by western blot (figure 6B). We then compared GD3s and GD2s expression between p53 k/d cells and shRNA controls and found no significant difference (figure 6C). Similarly, there is no significant difference in the percentage of $GD3^+$ cells measured by flow cytometry (97.4% vs 100%) (figure 6D). These results indicate that the specific hotspot p53 mutation R273H expressed in MDA-MB-468 cells doesn't provide a survival advantage and does not play a role in the regulation of the GD2 biosynthesis pathway. Because the majority of cell lines with hotspot mutations were heavily dependent on mutant p53 for survival, we were not able to harvest enough cells to examine the effect of mutant p53 knockdown on TNBC BCSCs function in vitro and in vivo.

A)

Cell line	Stable p53 knockdown	Transient p53 knockdown	Dependency on p53	Effect on GD3	Effect on GD3s	Effect on GD2s	Effect on GD2
HCC70	Yes	Yes	Cells died following stable knockdown	N/A	N/A	N/A	N/A
MDA 468	Yes	Yes	Cells survived following stable knockdown	No effect	no significant change	no significant change	No effect
BT549	Yes	No	Cells died following stable knockdown	N/A	N/A	N/A	N/A
Hs578T	Yes	No	Cells died following stable knockdown	N/A	N/A	N/A	N/A
HCC38	Yes	No	Cells died following stable knockdown	N/A	N/A	N/A	N/A
HCC1395	Yes	No	Cells not growing well after knockdown	N/A	N/A	N/A	N/A

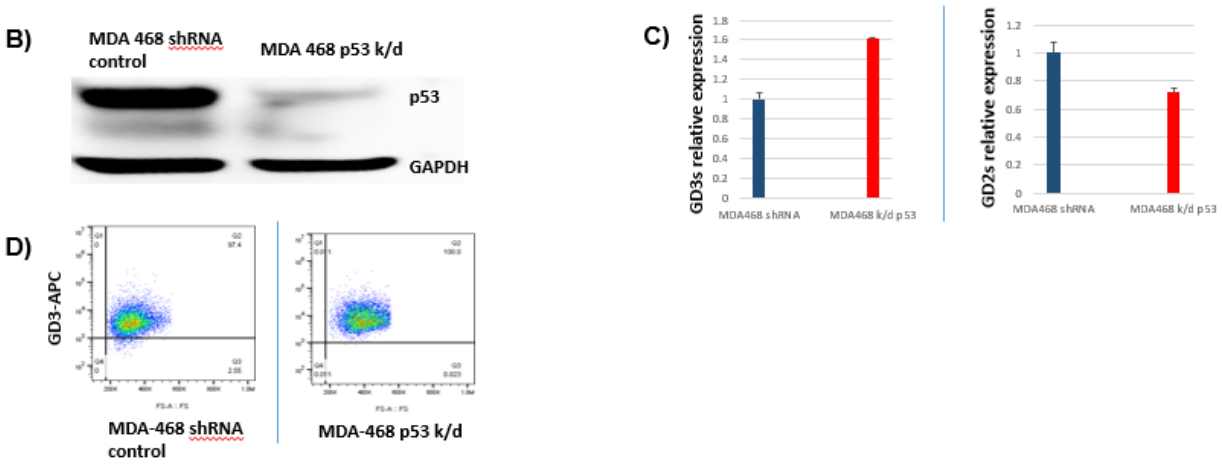

Figure 6: (A) Lenti-viral mediated stable knockdown of mutant p53 was performed in 6 available TNBC cell lines with hotspot p53 mutations. We noted the dependency of each cell line on mutant p53 for survival. **(B)** Mutant p53 knockdown efficiency was validated by western blotting in MDA-MB-468 cells in comparison to shRNA transfected controls. **(C)** RNA extraction was performed by ethanol precipitation. GD3 synthase and GD2 synthase expression was measured by RT-qPCR in p53 knockdown MDA-MB-468 cells compared to shRNA controls. **(D)** GD3 expression was measured by flow cytometry in p53 knockdown MDA-MB-468 cells compared to shRNA controls

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

This project provided a great learning and training opportunities for two postdoctoral fellows and a summer training student to learn and establish drug discovery station in lab and conduct computational modeling. It also gave exposure to the concepts of homology modeling, virtual screening and *in silico* analysis. In addition, this project provided lab members an opportunity to gain more knowledge about p53 mutations and their role in regulating TNBC BCSCs functions. It also allowed lab members to enhance their knowledge and technical skills through performing several experiments.

How were the results disseminated to communities of interest?

The results from this study are frequently discussed with breast cancer research communities especially with Breast Medical Oncology department at MD Anderson Cancer Center. An abstract entitled "Hotspot p53 mutations correlate with increased expression of stem cell markers in triple negative breast cancer" was submitted to the San Antonio Breast Cancer Symposium 2020, as is currently under review. A manuscript will be submitted upon completion of the study.

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

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

1. We plan to identify additional inhibitors from FDA approved compounds and assess their effects in vitro and in vivo. As these will be first generation drugs, structural modifications need to be performed to improve their affinity and efficacy. Then the compounds will be tested for their effect on tumorigenesis and metastasis.
2. Identify all hotspot p53 mutations that regulate GD3s expression.
3. Measure GD3s and GD2 in TNBC frozen sections by IHC, and make correlations with p53 mutation status and other clinical parameters to validate our findings in patient samples
4. Selectively knockdown specific hotspot p53 mutant forms using FANA ASO sequences without affecting wild type p53 function. Evaluate the efficacy of this therapeutic approach in mice models
5. Identify the promoter sequence to which mutant p53 binds using chromatin immunoprecipitation to provide a mechanistic explanation of mp53's role in the regulation of GD3s expression
6. IHC analysis of GD2 and GD3 synthase in TNBC tumor sample and its clinical correlation with the disease status and TNBC subtyping into distinct molecular groups characterized by unique gene expression.
7. Determine the effect of ST8SAI1 inhibition on mitophagy and autophagy in different human breast cancer cell lines.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
Since >70% of patients with TNBC carry mutation in TP53 gene it is important to understand the role of these mutations on cancer stem cells function. Our data demonstrate that some of the TP53 mutations especially the hotspot mutations regulate GD2 and GD3 synthase expression.

What was the impact on other disciplines?
Since GD2 and GD3 synthase are also expressed in other cancer types including neuroblastoma, melanoma and osteosarcoma, new findings in this study could potentially have an impact on other tumor types.

What was the impact on technology transfer?
Nothing to report.

What was the impact on society beyond science and technology?
Nothing to report.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.
Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Due to the COVID-19 pandemic, a 3-month lab shutdown was imposed between March 2020 and June 2020. Subsequently, the labs were allowed to reopen with partial resumption of experiments during working hours in shifts. This led to a delay in conducting experiments and generating data relevant to this project. As a precautionary measure, the laboratories will be permitted to fully re-open in October 2020. To utilize the time more efficiently during the pandemic, we competed some of the tasks (including the role of TP53 mutations TNBC) that were supposed to be performed in year 2. However, we are trying to achieve our goal in the stipulated time-frame.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.
We delay with recruitment of one of the Postdoctoral fellow. This significantly impacted the expenditures on this project.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Due to the COVID-19 pandemic experiments associated with human tissue samples, vertebrate animals and specific GD3 synthase inhibitors were significantly delayed.

Significant changes in use or care of human subjects.

Significant changes in use or care of vertebrate animals.

Significant changes in use of biohazards and/or select agents.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  An abstract entitled "Hotspot p53 mutations correlate with increased expression of stem cell markers in triple negative breast cancer" was submitted to the San Antonio Breast Cancer Symposium 2020, as is currently under review.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	*Dr. Prashen Chelikani* *Professor, University of Manitoba, Canada*
Project Role:	*Molecular modeling consultant and software support*
Researcher Identifier (e.g. ORCID ID):	*ORCID:*0000-0003-1129-795

Contribution to Project:	*Dr. Chelikani involves in project update meetings and provide expert guidance with respect to the identification of GD3S inhibitors. We have access to their modeling suite and used for virtual ligand screening*

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

Dr. Battula - Nothing to report.

Dr. Ueno – Nothing to report.

Dr. Piwnica-Worms – New

RP200120 (H. Piwnica-Worms) 03/01/20 – 02/28/23 3 CM (25%)
Cancer Prevention Research Institute of Texas (CPRIT) IIRA $285,000/year
Single-cell evaluation to identify tumor-stroma niches driving the transition from in situ to invasive breast cancer
The goal of these proposed studies is to evaluate the relationship between DCIS tumor cells and their neighboring non-tumor cells to better understand how the communication between the DCIS and the non-tumor cells promote aggressive disease.
Role: PI

Dr. Sahin – New

BC180196 (Schneekloth) 9/1/2019-8/31/2022 0.24 Calendar
Department of Defense (DOD) $1,200,000
Molecular Mechanism Elucidation and Therapeutic Strategies against TNBC Chemotherapy-Induced Metastasis
The goal of this study is to identify the mechanism underlying chemotherapy-induced metastasis which could be attenuated by combinatorial therapy.
Role: Co-Investigator

Dr. Peterson – New

Title/Grant Number:	Patient-centered liver cancer prevention in the Houston community / RP190513
Effort:	0.6 calendar months, 5% effort
Supporting Agency:	Cancer Prevention & Research Institute of Texas (CPRIT)
Grants Officer:	Patricia Moore, cprit@cprit.state.tx.us
Performance Period:	8/31/2019-8/30/2024
Funding Amount:	$474,704
Project Goals:	To develop a comprehensive assessment to efficiently identify persons with risk factors for fibrosis and cirrhosis in the community clinic setting. PID8181
Specific Aims:	To develop a comprehensive assessment to efficiently identify persons with risk factors for fibrosis and cirrhosis in the community clinic setting, and 2. To determine the effectiveness of a behaviorally-based tailored disease

	management intervention in patients with risk factors for fibrosis and cirrhosis.
Overlap:	None
Title/Grant Number:	Targeting Breast Cancer Stem Cells / BCRF-18-007
Effort:	0.24 calendar months, 2% effort
Supporting Agency:	Breast Cancer Research Foundation
Grants Officer:	Dorray El-Ashry, 646-497-2626, grants@bcrf.or
Performance Period:	10/1/2019-9/30/2020
Funding Amount:	$208,335
Project Goals:	To inhibit conversion of glutamine to glutamate by inhibiting glutaminase targets BCSC function and to test activity of the NRF2 inhibitor brusatol in inhibition of tumor initiation and metastasis in vivo. PID8204
Specific Aims:	1) Determine the role of glutamine in biosynthesis of ganglioside GD2 in TNBC cells. 2) Targeting glutaminase (GLS) to inhibit BCSCs using small molecule inhibitors. 3) Determine the role of NRF2 in GD2+ BCSC function in vivo using PDX models. 4) Conduct Phase-1 clinical trial using chimeric anti-GD2 monoclonal antibody (Dinutuximab) in combination with chemotherapy in metastatic TNBC patients.
Overlap:	None
Title/Grant Number:	Rational Combinations to Target the Replication Stress Response in Prostate Cancer / FP00007799
Effort:	0.6 calendar months, 5% effort
Supporting Agency:	Prostate Cancer Foundation
Grants Officer:	info@pcf.org, Phone: (800) 757-2873
Performance Period:	10/1/2019-9/30/2022
Funding Amount:	$75,000
Project Goals:	To test the hypotheses that novel transcriptomic and proteomic biomarkers of RSR deficiency will predict response to DNA damaging agents and DDR inhibitors. PID8359
Specific Aims:	1) To determine if select biomarkers of RSR defects predict response to platinum-based chemotherapy and/or PARP inhibition; and, to analyze dynamic changes in the RSR pathway as a product of drug treatment in mCRPC. 2) To perform a Phase I/II clinical trial of ATR inhibitor (BAY1895344) in combination with second generation androgen blockade (darolutamide) in patients with metastatic prostate cancer. 3) To interrogate the impact of ATR kinase inhibitor based treatment on innate immune activation and tumor immune microenvironment in patients and immunocompetent preclinical models of metastatic prostate cancer.
Overlap:	None

Title/Grant Number:	SIV-Varian Strategic Alliance Project: Networked Plan QA & Review Platform / 57688
Effort:	0.6 calendar months, 5% effort
Supporting Agency:	Varian Medical System Foundation
Grants Officer:	3100 Hansen Way, M/S E-190 Palo Alto, CA 94304
Performance Period:	10/7/2019-10/6/2024
Funding Amount:	$2,000,000
Project Goals:	Build and deploy a cloud-based platform for network plan QA & review by MDACC division of radiation oncology. (Effort Effective 5/1/20) PID11263
Specific Aims:	1) Develop and deploy a "platform" based cloud solution for the plan QA & review services provided to the mdacc affiliate network. 2) Develop machine learning and ai based decision support tools for plan qa & review applications using mdacc data.
Overlap:	None

Title/Grant Number:	Stop LCNP: High dose steroid therapy for late radiation-associated lower cranial neuropathy / AWD00004455
Effort:	0.12 calendar months, 1% effort
Supporting Agency:	Health and Environmental Sciences Institute
Grants Officer:	hesi@hesiglobal.org, 202-659-8404
Performance Period:	1/1/2020-12/31/2021
Funding Amount:	$49,925
Project Goals:	To use high dose steroid therapy to improve swallowing and speech function in long-term survivors of head and neck cancer (HNC) who have nerve dysfunction as a late side effect of their cancer treatment. PID11019
	Specific Aims:Aim 1: Establish feasibility and optimal dosing of steroid therapy in HNC survivors with late LCNP
	Aim 2: Examine endpoints that may be sensitive to functional or symptomatic gains with steroid therapy for late LCNP
Overlap:	None

Title/Grant Number:	Artificial intelligence for the peer review of radiation therapy treatments / RP200395
Effort:	0.6 calendar months, 5% effort
Supporting Agency:	Cancer Prevention & Research Institute of Texas (CPRIT)
Grants Officer:	Patricia Moore, 512-305-8491, pmoore@cprit.texas.gov
Performance Period:	3/1/2020-5/31/2023
Funding Amount:	$285,000
Project Goals:	To create an automated solution for review of contours and treatment plans, and to test this system against our current manual process. PID11229 (Effort effective 6/1/20)
Specific Aims:	Aim 1: Create an automated peer-review system to evaluate contours for radiation therapy treatment plans.
	Aim 2: Create an automated peer-review system to evaluate the quality of radiation therapy treatment plans.
	Aim 3. Assess the impact of CASPER in clinical practice.

Overlap:	None
Title/Grant Number:	Targeting the Replication Stress Response to Induce Anti-Cancer Immunity in Advanced Solid Malignancies / AWD00004826
Effort:	0.6 calendar months, 5% effort
Supporting Agency:	Conquer Cancer Foundation
Grants Officer:	(571) 483-1700, grants@conquer.org
Performance Period:	7/1/2020-6/30/2023
Funding Amount:	$62,715
Project Goals:	To target the replication stress response to induce anti-cancer immunity in advanced solid malignancies. PID11495
Specific Aims:	1) Determine the clinical safety and efficacy of ATR inhibition in combination with immune checkpoint blockade in patients with advanced solid cancers and DNA damage response (DDR) aberrations. 2) Determine if a transcriptomic signature of replication stress response deficiency and/or loss of ATM protein expression in patient tumors can serve as functional predictive biomarkers of response to ATR inhibition and immune checkpoint blockade. 3) Determine how ATR inhibition impacts innate immune activation, as well as adaptive immune cell and metabolic changes in the tumor microenvironment in patients with advanced genitourinary malignancies and associated preclinical models9.24.19- email Sandra for overall goals and SA.
Overlap:	None

Title/Grant Number:	Protecting colonic mucus to mitigate acute intestinal graft-versus-host disease
Effort:	0.6 calendar months, 5% effort
Supporting Agency:	NIH/NHLBI
Grants Officer:	Lisbeth Welniak, 301-435-0073, welniakla@nhlbi.nih.gov
Performance Period:	7/1/2020-6/30/2025
Funding Amount:	$558,580
Project Goals:	To develop strategies that will change standard of care for prophylaxis and treatment of this major cause of morbidity and mortality in this patient population. PID11626
Specific Aims:	Aim 1: In mouse models, we will quantify mucus degradation during acute GVHD and evaluate promising strategies to target mucus degradation, including antibiotic and metabolic strategies. Aim 2: In allo HCT patients, we will characterize the contribution of mucus degradation in the pathophysiology of acute intestinal GVHD.
Overlap:	None

What other organizations were involved as partners?
Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*