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Official Study Title:
AGING MAMMARY STEM CELLS AND BREAST CANCER PREVENTION

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PROJECT TITLE: AGING MAMMARY STEM CELLS AND BREAST CANCER PREVENTION

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DATE: July 21, 2020

INVESTIGATOR'S AGREEMENT

I have read and understand the contents of this clinical protocol, UT Health San Antonio Mays Cancer Center CTMS# 15-2096, and will adhere to the study requirements as presented, including all statements regarding confidentially. In addition, I will conduct the study in accordance with current international conference on harmonization (ICH) guidance, Good Clinical Practice (GCP) guidance, the Declaration of Helsinki, US Food and Drug Administration (FDA) regulations and local IRB and legal requirements.

Name of Clinical Investigator: Ismail Jatoi, MD

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Investigator Signature Date

ABBREVIATIONS

3D-ECM	3D Extracellular Matrix of Matrigel
ADH	Atypical Ductal Hyperplasia
AE	Adverse Event
ALH	Atypical Lobular Hyperplasia
BP	Biological Processes
DAVID	Database for Annotation, Visualization and Integrated Discovery
DCIS	Ductal Carcinoma In Situ
DSM	Data Safety Monitoring
DSMB	Data Safety Monitoring Board
DSMC	Data Safety Monitoring Committee
DSMP	Data Safety and Monitoring Plan
DSO	Data and Safety Officer
DQA	Director of Quality Assurance
ECOG	Eastern Cooperative Oncology Group – Performance Status
ERα	Estrogen Receptor Alpha
eRapa	Microencapsulated Rapamycin
FDR	False Discovery Rate
GCP	Good Clinical Practice
GO	Gene Ontology
GSEA	Gene Signature Enrichment Analysis
IBC	Invasive Breast Cancer
IND	Investigational New Drug
LCIS	Lobular Carcinoma In Situ
MaSC	Mammary Stem/Progenitor Cells
MEC	Mammary Epithelial Cells
NIA	National Institute of Aging
QAD	Quality Assurance Division
PALS	Priority of Audit Level Score
PI	Principal Investigator
SAE	Serious Adverse Event
SASP	Senescence Associated Secretory Phenotype
SFD	Sphere Formation and Differentiation
SIR	Senescence Associated Inflammatory Response
UPIRSO	Unanticipated Problem Involving Risks to Subjects or Others

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1. SYNOPSIS

Title	**Aging Mammary Stem Cells and Breast Cancer Prevention**
Design	Non-randomized, open-label, phase II, window of opportunity trial.
Objectives	The overall purpose of this study is to determine if rapamycin can reduce malignant markers and MaSC number in surgical specimens. The primary objectives are: 1. Reduction of MaSC in DCIS, LCIS, ALH or ADH in patients receiving rapamycin 2. Reduction of malignant markers in DCIS, LCIS, ALH or ADH in patients receiving rapamycin The secondary objectives are: 1. Toxicity 2. Surgical complications
Patient Population	Women (pre and post-menopausal) with DCIS, LCIS, ALH or ADH undergoing surgical resection.
Treatment Plan	Women who consent will be given rapamycin for 5-7 days treatment at 2 mg/day. Patients will undergo mastectomy or lumpectomy 3-7 days after the last dose of rapamycin. Pathological and molecular biomarkers associated with breast cancer aggressiveness and recurrence will be assessed by the Breast Pathologist and the features of MaSCs will be determined by the PI's lab for determining the effect of rapamycin.
Efficacy Assessment	Efficacy will be measured by measurement of MaSC and malignant markers in tissue from patients with DCIS, LCIS, ALH or ADH

2. BACKGROUND AND SIGNIFICANCE

Aging – the No. 1 risk factor for breast cancer. Cancer incidence rises exponentially during midlife in humans and in mice[1]. About 80% of all breast cancers arise in women over age 50 and age specific increase of invasive breast cancer results in a cumulative lifetime risk of 13.2% or 1 in 8 (http://www.srab.cancer.gov/devcan). Even for women carrying BRCA1 and BRCA2 mutations with family history of familial breast cancer, aging is another important risk factor[2]. However, the knowledge regarding the cellular and molecular basis that links aging with clinical manifestation of breast cancer is indeed scanty[3-5]. Thus, understanding age-related changes, which promote breast cancer, should have a major impact on our ability to prevent and treat breast cancer for the high risk and the general aging population.

Does breast cancer originate from mammary stem cells? During aging, stem cells responsible for lifelong tissue maintenance and repair are susceptible to changes in their niche and genomic integrity. Recent evidences indicated that they may be responsible for neoplastic transformation. However, there is little direct experimental evidence linking aging mammary stem/progenitor cells to **spontaneous** pre-neoplastic transformation and tumorigenesis. Mammary stem cells (**MaSCs**) have been shown to have the potential to drive mammary gland development and to initiate neoplastic transformation when **genetically altered**[6-8]. Gene expression profiles of different subtypes of breast cancer have also been shown to correspond to the profiles of the basal or luminal mammary stem/progenitor cell-enriched epithelial cells, which suggests that MaSCs might be the origin of certain types of breast cancer. A recent study reported a shift in the balance between luminal and basal lineages in aging human breast cells and expansion of multipotent progenitor population with a basal differentiation bias[9]. These changes were postulated to contribute to aging-associated mammary tumorigenesis. As shown in our preliminary data below, we have demonstrated that aging is associated with increased number of aberrant MaSCs, which can generate mammary glands with **early neoplastic lesions**. Our study is the first experimental demonstration that MaSCs are the precursors of **spontaneous** neoplastic lesions and aging MaSCs appear to be predisposed to neoplastic transformation.

Role of aging/senescence-associated inflammatory response in tumorigenesis. Recent studies have suggested that senescent cells in the aged stroma might create a tissue microenvironment favoring cancer initiation and growth. Cellular senescence acts as a barrier to transformation, but it drives aging phenotypes and is also believed to induce a fertile ground for cancer development late in life[10]. Senescent cells increase with age in many mammalian tissues including human, primates and rodents[11,12] and are found at sites of many age-related pathologies including benign prostatic hyperplasia[13,14]. Senescent human and mouse cells secrete pro-inflammatory cytokines, chemokines, extracellular matrix components, and growth factors. A phenotype termed as senescence associated secretory phenotype (**SASP**) disrupts local tissue homeostasis and is believed to promote tumorigenesis.

Inoculation with premalignant or malignant epithelial cells together with senescent fibroblasts led to more and larger tumors in host mice than inoculation with the epithelial cells alone or with presenescent fibroblasts[15]. Using a *Cdkn2a*INK4A (p16)–luciferase reporter mouse model, one recent study found an exponential increase with age in senescence associated-expression of p16 and the induction of p16 in the benign stromal cells of the nascent neoplasm[16]. In our whole genome transcriptome analysis shown below, we found an inflammatory response both in the mammary stromal and MaSC-enriched cell compartments in association with senescent stromal cells. Although this senescence-associated inflammation response (**SIR**) has been shown in various tissues, it was only last year that SIR was shown

to contribute to colon tumorigenesis in genetically modified mouse models[17]. However, it is not known whether the SIR causes the transforming phenotype of MaSCs during progressive aging.

3. PRELIMINARY DATA

Development of a novel *in vitro* assay for quantifying MaSCs. In order to quantitatively measure the effect of aging on the number and function of murine mammary stem/progenitor cells, we recently developed a novel *in vitro* method[18]. Previously, murine MaSCs were quantified with limited dilution *in vivo* transplantation of MaSC-containing mammary epithelial cells into de-epithelized inguinal mammary glands (also denoted as cleared mammary fat pads) of 21-day-old mice and subsequent examination of regeneration frequency of a fully functional mammary gland. This *in vivo* regeneration method requires large number of recipient mice for transplantation and a relatively long period (8 weeks) of experiment turnover, not to mention the associated expensive cost. Our *in vitro* method, termed sphere formation and differentiation (**SFD**) assay, involves suspension culture of mouse MaSC-enriched basal cells, which are FACS-sorted out with their cell surface immunophenotype of Lin-CD49fhighCD24med (**Fig. 1A**)[19,20], for the formation of mammospheres (**Fig. 1B**). These spheres were verified to be of clonal origin of single MaSC[18], and they can be differentiated to form morphologically distinct solid 3D spherical structures/organoids when embedded in 3D extracellular matrix of Matrigel (3D-ECM) (**Fig. 1C**). Upon *in vivo* transplantation, these single mammospheres or 3D solid structures can generate full ductal-lobular outgrowths (**Fig. 1D**). We were able to visualize ductal structures of the regenerated mammary ducts with green fluorescence imaging (**Fig. 1D**) as the mammospheres and 3D solid structures were formed by MaSCs isolated from GFP-transgenic mice. Thus, this SFD assay allows quantification of MaSC *in vitro.*

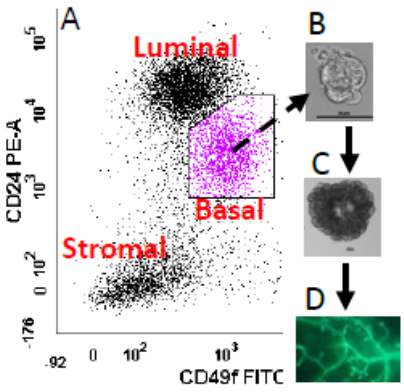

Fig. 1. *in vitro* and *in vivo* assays for mouse MaSC qualification and quantification. Primary cells were sorted with FACS (**A**), and used for the following assays. **Mammosphere assay (B)**: cells were cultured in a 96-well low attachment plate for 7 days in a mammosphere medium. **3D-ECM sphere differentiation assay (C)**: mammospheres were plated in Matrigel and cultured for 9 days to score 3D solid structures/organoids. ***in vivo* transplantation assay (D)**: single mammospheres or 3D solid structures were injected into cleared mammary fat pad of a 21-day-old recipient female mouse. After 8 weeks, regenerated mammary glands were visualized with green fluorescence imaging.

Age-associated changes in MaSC frequency and gene expression. Using this SFD assay, we measured MaSC number as a function of aging. FACS analysis revealed that total basal epithelial cell frequency, which is percent of basal epithelial cells in total lineage negative (**Lin-**) mammary epithelial cells (**MEC**) (**Fig. 1A**), increased with progressive aging in C57Bl/6 mice (**Fig. 2**). This aging-associated increase of basal epithelial cells and decrease of luminal epithelial cells (data not shown) led to a significant decrease in the luminal to basal epithelial cell ratio (**Fig. 3A**) and a significant increase in MaSC frequency (**Fig. 3B**) in old (22-30 month-old) mice of both C57Bl/6 and Balb/c strains when compared to their respective young (4-6 month-old) cohorts. However, the ability of the 3D structures to regenerated 3D structures during serial passage in 3D culture was much lower by the old MaSC-formed 3D structures than by the young MaSC-formed 3D structures (data not shown). To elucidate molecular changes associated with aging MaSCs, we

used RNA-seq to profile whole transcriptome of FACS-sorted basal, luminal, stromal cells, and early stage mammospheres formed by basal or luminal epithelial cells, which are highly enriched in MaSCs or luminal progenitors respectively, from young and old C57Bl/6 mice.

Using pairwise comparison among young mammary cells, we obtained a set of basal or luminal cell specific genes/markers, named Basal Signature or Luminal Signature respectively and performed gene signature enrichment analysis (**GSEA**) using the basal or luminal markers we identified as well as those previously reported by Lim et al.[21]. As shown in **Fig. 4**, the old basal (CD49fhighCD24med) cells and their mammospheres showed significantly reduced expression of basal markers, but significantly increased luminal markers. In contrast, GSEA did not show a significant change of the basal or luminal signatures in old luminal (CD49flowCD24high) cells and their mammospheres (data not shown). These findings indicate aberrant changes of old MaSCs towards a more luminal phenotype, which may contribute to their reduced self-renewal capacity and transforming property as described below.

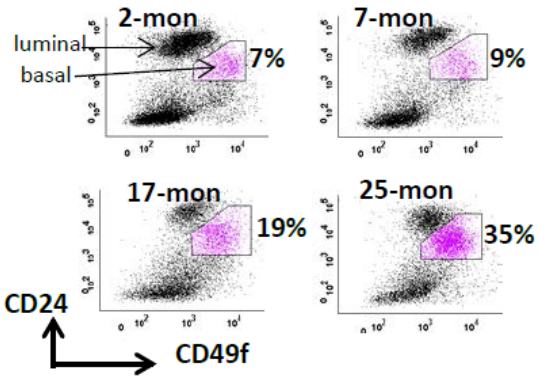

Fig. 2. Representative FACS profiles of Lin⁻ MEC (luminal+basal fraction) from C57Bl/6 mice at different ages showing basal MaSC-enriched fraction (purple).

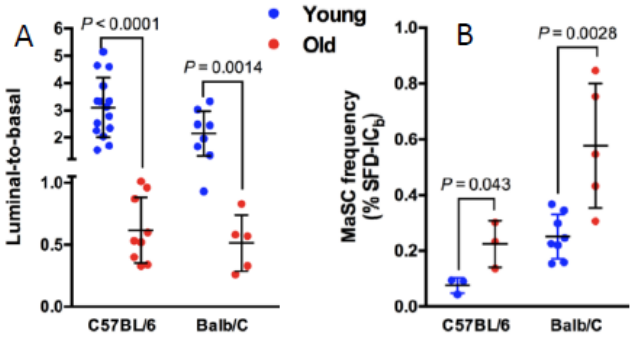

Fig. 3. Aging-associated changes in (A) luminal to basal epithelial cell ratio and (B) MaSC frequency in young and old female C57Bl/6 and Balb/c mammary glands. Each dot is one mouse. P values were from Student *t* tests.

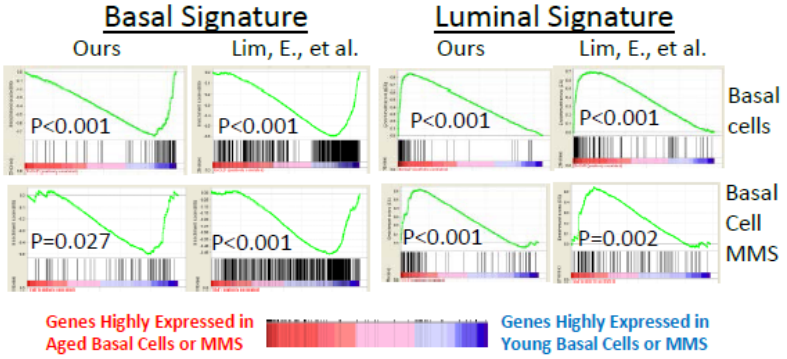

Fig. 4. Gene signature enrichment analysis show that old CD49f^{high} basal cells and their mammospheres (MMS) have reduced expression of basal markers derived from our RNA-seq or Lim et al, *Breast Cancer Res* **12**:R21, 2010, but have enriched luminal markers in comparison with young basal cells and their MMS respectively.

Old MaSC-mediated development of spontaneous neoplastic lesions. Significantly, *in vivo* transplantation of single 3D solid structures and subsequent histology analysis by a breast pathologist revealed that the ducts of the old MaSC-regenerated mammary glands were similar to the ducts of the old primary mammary glands with significantly higher number of hyperplastic and dysplastic foci of atypical ductal hyperplasia (**ADH**, indicated by abnormal glandular proliferation) and ductal carcinoma in situ (**DCIS**, indicated by numerous mitotic figures, nuclear enlargement, and coarse chromatin pattern) than those from the young primary glands and young MaSC-regenerated glands (**Fig. 5A-C**). We define the ability of generating the early neoplastic lesions in the MaSC-regenerated glands as the transforming phenotype/property of MaSCs. Interestingly, the cells in the dysplastic lesions of the old MaSC-regenerated glands express various luminal epithelial markers such as estrogen receptor alpha (**ERα**) and Her2 (**Fig. 6**) indicating their resemblance to human breast adenocarcinoma.

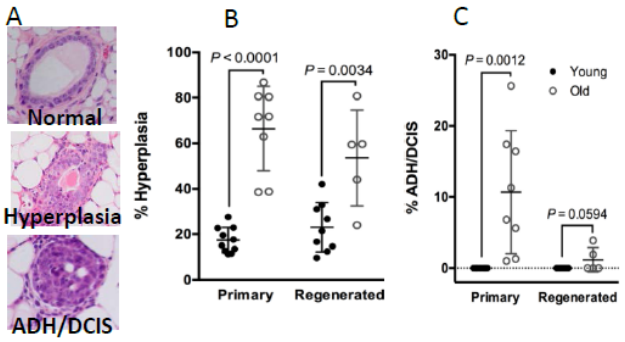

Fig. 5. Early neoplastic lesions in the primary and MaSC-regenerated glands. Primary and MaSC-regenerated mammary glands were processed for sectioning and H&E staining from each mouse. Normal, hyperplasic, and dysplastic (ADH: atypical ductal hyperplasia; DCIS: ductal carcinoma *in situ*) ducts (Panel A) in H&E stained slides were counted and presented as percent of total ducts in a given tissue section slide. Percent of hyperplastic and dysplastic ducts in sections of primary glands of young (solid dot) and old (circle) mice and their MaSC-regenerated glands is shown in Panels B and C. Each dot represents data from one mouse. P values were obtained with Student *t* tests.

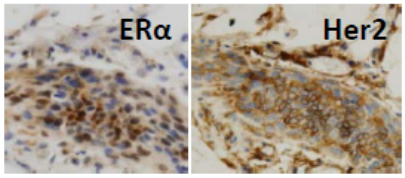

Fig. 6. Old MaSC-regenerated mammary neoplastic lesions in C57Bl/6 mice expressing ERα and Her2. The brown color depicts positive staining of ERα or Her2

Increased inflammation and immune responses in old mammary cells. As the initial step in elucidating cellular pathways that may contribute to the aging-associated transforming phenotype of MaSCs, we compared gene expression profiles between young and old mammary stromal cells and found that the *Cdkn2a* locus, containing p16Ink4a and p19Arf, was the most highly up-regulated transcript in the old stromal cells among all significantly (adjusted P value (aka **FDR**, false discovery rate)<0.05) and differentially regulated genes with a 19-fold difference (see the heatmap in **Fig. 10**). The significant increase of both p16Ink4a and p19Arf in the old stromal cells was confirmed with real time RT-PCR (**Fig. 7**) suggesting a senescence phenotype of the old stromal cells. This is consistent with the old mammary gland expressing higher senescence-associated β-gal activity (**Fig. 8**). We found 66 differentially expressed genes between young and old stromal cells with an adjusted P value of <0.05 (**Fig. 9**).

Functional annotation of these differentially expressed genes with the Database for Annotation, Visualization and Integrated Discovery (**DAVID**)[22] platform identified many significantly (P<0.05) altered Biological Processes (**BPs**) depicted as gene ontology (**GO**) terms with the top ten listed in **Fig. 9**. While over 200 genes were found to be differentially expressed between young and old luminal progenitor-enriched spheres, DAVID analysis yielded top enriched BPs mostly related to cell adhesion, biological adhesion, and extracellular matrix organization (data not shown) suggesting that aging-associated luminal cell functions are more involved in epithelium structure remodeling with a limited role in the early neoplastic transformation by the old MaSCs. Consequently, we have focused our attention on the altered gene expression in aging stromal cells and MaSCs. Although there are only 7 genes that are differentially regulated during aging in both basal cell-formed mammospheres and stromal cells (**Fig. 9**), the functional annotation revealed that the top enriched Biological Processes in both sets of the genes are related to immune, inflammatory, defense, and wounding responses (**Fig. 9**).

These processes and responses are mostly performed by a shared set of genes in each cell type, which are shown in the heatmaps in **Fig. 10**. Some of the genes associated with the Oxidation Reduction, the top GO term for the stromal gene set, are involved in biosynthesis/production of eicosanoids, which may also contribute to inflammation (**Fig. 10**). We have also observed a significant increase of *Ptgs2* transcript in our RNA-seq data (pointed by the arrow in **Fig. 10**) and its protein COX2 in old mammary gland with immunostaining (**Fig. 14** shown in Aim 2.2), and an increased number of infiltrating macrophages in the old mammary gland (data not shown). Thus, there is an increased senescence-associated inflammation response (**SIR**) in the old mammary glands.

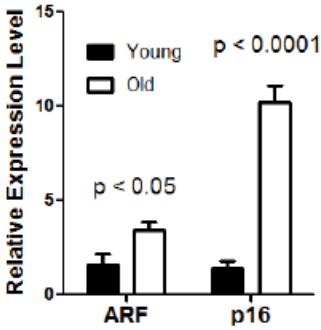

Fig. 7. RNA levels in stromal cells with real time RT-PCR. Each bar is mean±SEM of three replicate measurements of two mice.

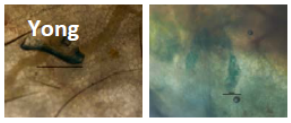

Fig. 8. Representative senescence-associated β-Gal stained tissues of young and old mammary gland. Blue staining indicates high senescence-associated β-gal activity.

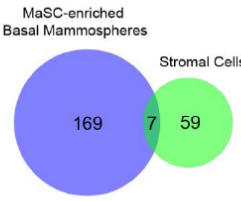

Fig. 9. Venn diagram and top ten GO terms from DAVID. The P values indicating the probability of no functional association of each GO term with the differentially expressed gene set is shown next to each GO term.

Immune Response	4.91E-09
Defense Response	1.56E-06
Response to Wounding	3.28E-05
Cell Adhesion	1.07E-04
Biological Adhesion	1.09E-04
Epithelium Development	3.11E-04
Inflammatory Response	3.47E-04
Innate Immune Response	6.08E-04
Acute Inflammatory Response	1.14E-03
Negative Regulation of Cell Proliferation	6.24E-03

Oxidation Reduction	1.72E-04
Inflammatory Response	6.15E-04
Response to Wounding	6.41E-04
Pyruvate Metabolic Process	2.67E-03
Defense Response	1.19E-02
Immune Response	1.45E-02
Hormone Catabolic Process	1.54E-02
Positive Regulation of Response to Stimulus	1.96E-02
Acute Inflammatory Response	2.59E-02
Fatty Acid Biosynthetic Process	2.59E-02

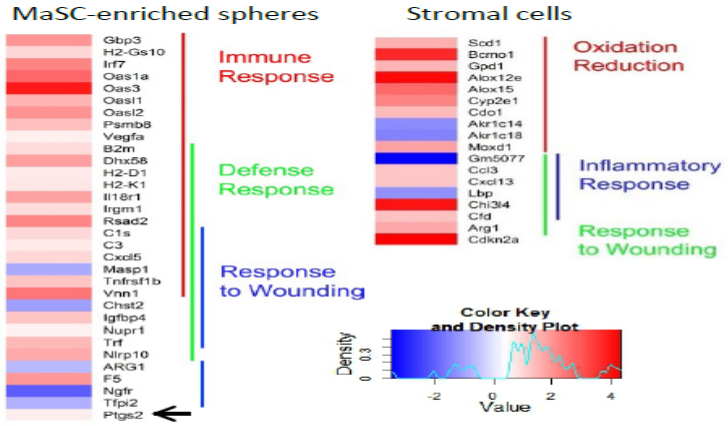

Fig. 10. Heatmap of the fold change of significantly (FDR<0.05) differentially expressed genes associated with senescence and top three GO terms shown in Fig. 6 between young and old mammosphere and stromal cells. The genes associated with the three GO terms are marked with the colored vertical bars. Heatmap colors indicate the logarithm of the fold change.

Rapamycin treatment mitigated the aberrant phenotypes of old MaSCs. To investigate whether the increased SIR in the old mammary gland contributes to the increased number and transforming phenotype of the old MaSCs, we tested the efficacy of rapamycin in inhibiting the aging-associated mammary gland dysfunction because it is an anti-inflammatory and immune modulatory drug and its target mTOR is known to be up-regulated in senescent cells[23]. Rapamycin was shown to significantly extend lifespan and reduce tumor incidence in mice by investigators in our university as well as by others[24]. It was also shown recently to inhibit senescent mammary stroma-promoted growth of

xenografted breast cancer cells in a premature aging mouse model with *Cav-1* knockout[25]. As shown in **Fig. 3B**, old mammary glands have higher percent of MaSCs. Both long-term (2 years) and short-term (5-10 days) treatment with a diet containing eRapa (microencapsulated rapamycin) significantly reduced the frequency of MaSCs in old mice (**Fig. 11A**). More significantly, both long- and short-term treatment also reduced hyperplastic and dysplastic foci not only in the primary old mammary glands but also in the regenerated glands by old MaSCs (**Fig. 11B** and **C**).

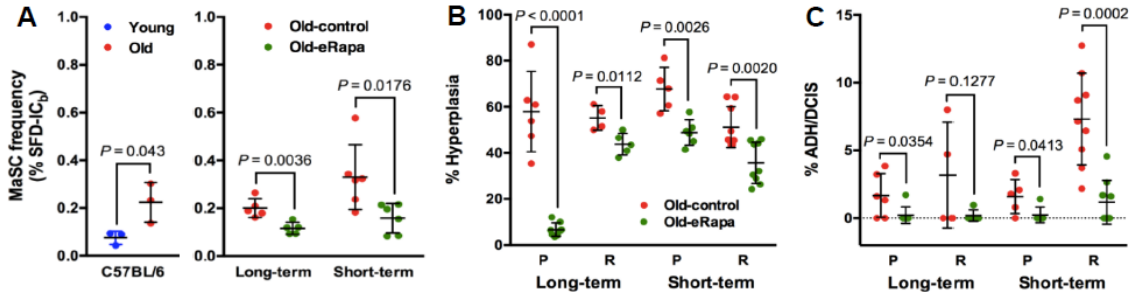

Fig. 11. Decreased MaSC frequency and early neoplastic foci after rapamycin treatment. Frequency (mean±SD) of MaSC (A) expressed as percent sphere formation and differentiation initiating cell in the basal cell fraction (%SFD-IC$_b$) in young (blue dots) and old (red dots) mammary glands from C57Bl/6 (2 vs. 26 months), and in long-term (2 yr) and short-term (5-10 day) encapsulated rapamycin (eRapa)-treated (green dots) or control (red dots) old C57Bl/6 mice (26 months of age upon termination). The frequency of pre-neoplastic (expressed as %hyperplasia, B) and neoplastic (%ADH/DCIS, C) lesions in primary (P) and MaSC-regenerated glands (R) of long-term and short-term eRapa-treated (green dots) or control (red dots) mice (26 months of age). Each dot in the plots represents one mouse.

These exciting novel observations prompted us to propose the following research aims.

4. OBJECTIVES

The overall purpose of the study is to evaluate the efficacy of short-term rapamycin treatment on malignant markers and MaSC in patients with DCIS, LCIS, ALH or ADH.

4.1 Primary Objective

1. Reduction of MaSC in DCIS, LCIS, ALH or ADH in patients receiving rapamycin
2. Reduction of malignant markers in DCIS, LCIS, ALH or ADH in patients receiving rapamycin

4.2 Secondary Objectives

1. Toxicity
2. Surgical complications

5. PATIENT ELIGIBILITY

5.1 Inclusion Criteria

Subjects must meet all of the following criteria to be eligible for the trial:

1. Women with confirmed menopausal status. All patients who have NOT had a prior bilateral oophorectomy and/or are younger than age 60, will require menopausal status verified by FSH and estradiol local labs.
3. Women diagnosed with DCIS, LCIS, ALH or ADH lesions detected by pathology
4. Women scheduled for mastectomy or lumpectomy after DCIS, LCIS, ALH or ADH diagnosis
5. Women consented to the tissue biorepository (HSC20070684H/CTRC# 07-32)

6. Women of child-bearing potential willing to practice 2 forms of contraception, one of which must be a barrier method until at least 30 days after the last dose of rapamycin.
7. Women of child-bearing potential must have a negative serum pregnancy test at time of enrollment.
8. Patients must be able to swallow and retain oral medication.
9. All patients must have given signed, informed consent prior to registration on study.
10. Patients must have normal organ and marrow function as defined below:
    a. Leukocytes ≥ 3,000/μL
    b. Absolute neutrophil count **≥** 1,500/μL
    c. Platelets ≥ 100,000/μL
    d. AST ≤ 2.5 X ULN
    e. ALT ≤ 2.5 X ULN
    f. Total bili ≤ 1.5 X ULN or Direct bili ≤ 1 X ULN

5.2 Exclusion Criteria

1. Women who are pregnant.
2. Women who are receiving any other concomitant treatment for their DCIS, LCIS, ALH or ADH
3. Women who are taking rapamycin for another diagnosis.
4. Women with an allergy to rapamycin or its derivatives.
5. Active infection requiring systemic therapy.
6. Patients who are taking any pills containing herbal (alternative) medicines are NOT eligible for participation. Patients must be off any such medications by the time of registration.
7. Immunocompromised subjects, including patients with human immunodeficiency virus
8. Women currently taking strong CYP3A4 inducers or inhibitors. Drugs that cannot be coadministered with rapamycin include but are not limited to: Calcium channel blockers: nicardipine, antifungal agents: clotrimazole, fluconazole, antibiotics: troleandomycin, rifapentine, gastrointestinal prokinetic agents: cisapride, metoclopramide, Other drugs: bromocriptine, cimetidine, danazol, HIV-protease inhibitors (e.g., ritonavir, indinavir), anticonvulsants: carbamazepine, phenobarbital, phenytoin. *Appendix 4* has a complete list of these medications.
9. Patients with any of the following conditions or complications are NOT eligible for participation:
    a. GI tract disease resulting in an inability to take oral medication
    b. Malabsorption syndrome
    c. Require IV alimentation
    d. History of prior surgical procedures affecting absorption
    e. Uncontrolled inflammatory GI disease (e.g., Crohn's, ulcerative colitis)

6. TREATMENT PLAN

This is a phase II window of opportunity trial in which women with a diagnosis of DCIS, LCIS, ALH or ADH will be treated with rapamycin at 2 mg/day for 5-7 days. Surgery will be performed 3-7 days after the last dose of rapamycin. An evaluable patient for toxicity is any patient who has received at least 1 dose of rapamycin. An evaluable patient for efficacy is any patient who has completed at least 5 days of rapamycin therapy and has had surgery within the 7-day window.

7. STUDY DRUG ADMINISTRATION

Study drug will be given orally once a day in the form of tablets. Patient may take drug consistently with or without food.

8. TOXICITIES AND ADVERSE REACTIONS

Rapamune®
The incidence of adverse reactions was determined in two randomized, double-blind, multicenter controlled trials in which 499 renal transplant patients received Rapamune Oral Solution 2 mg/day, 477 received Rapamune Or l Solution 5 mg/day, 160 received azathioprine, and 124 received placebo. All patients were treated with cyclosporine and corticosteroids. Data (≥12 months post-transplant) presented in the table below show the adverse reactions that occurred in any treatment group with an incidence of ≥20%.

Specific adverse reactions associated with the administration of Rapamune (sirolimus) Oral Solution occurred at a significantly higher frequency than in the respective control group. For both Rapamune Oral Solution 2 mg/day and 5 mg/day these include hypercholesterolemia, hyperlipemia, hypertension, and rash; for Rapamune Oral Solution 2 mg/day acne; and for Rapamune Oral Solution 5 mg/day anemia, arthralgia, diarrhea, hypokalemia, and thrombocytopenia. The elevations of triglycerides and cholesterol and decreases in platelets and hemoglobin occurred in a dose-related manner in patients receiving Rapamune.

Patients maintained on Rapamune Oral Solution 5 mg/day, when compared with patients on Rapamune Oral Solution 2 mg/day, demonstrated an increased incidence of the following adverse events: anemia, leukopenia, thrombocytopenia, hypokalemia, hyperlipemia, fever, and diarrhea. In general, adverse events related to the administration of Rapamune were dependent on dose/concentration.

9. DOSE MODIFICATIONS

No dose modifications will be allowed.

10. CONCOMMITANT MEDICATIONS

All concomitant treatments, including blood and blood products, must be reported on the appropriate electronic case report form (eCRF).

10.1 Not Permitted
Patients who are taking any herbal (alternative) medicines are NOT eligible for participation. Patients must be off any such medications before the first dose of study treatment. Patients who are undergoing concomitant radiotherapy or chemotherapy are NOT eligible for participation.

10.2 Permitted
Supportive care, including but not limited to anti-emetic medications, may be administered at the discretion of the treating physician.

11. DURATION OF THERAPY

Treatment duration is 5-7 days

12. FOLLOW-UP POST-THERAPY

Patients will be followed 3 months after surgery at the time of routine clinic visit or via phone call with study personnel, See APPENDIX 3 –subsection titled Research Chart (case report form needed for data collection during this 3-month follow-up visit).

Treatment after study completion will be left to the discretion of the treating physician(s) and will not be considered part of this study. However, patients should still be followed for surgical complications as part of their standard of care.

13. SCHEDULE OF EVENTS

Procedures	Screening & Baseline	Day 1-7[4]	Day before Surgery	Surgery[2]	3 month Follow-Up
Tissue procurement[1]				X	
Informed consent	X				
Rapamycin administration		X			
Cancer history[3]	X		X		
Physical exam[3]	X		X		
CBC w/ diff	X[3]		X		
Comprehensive metabolic panel	X[3]		X		
Lipid panel	X		X		
Estradiol[5]	X		X		
FSH[5]	X		X		
Toxicity (AE) assessment			X		X

[1] Fresh tissue procurement will be required for study purposes.

[2] Surgery can take place 3-7 days after completion of rapamycin treatment

[3] Pre-study laboratory tests, cancer history, and physical exam (including review of systems) must be completed within 28 days prior to registration. At the discretion of the treating physician and only if clinically indicated as part of their standard of care, the pre-study CBC and CMP will be repeated one (1) day prior to treatment administration.

[4] Rapamycin will be administered for 5-7 days

[5] Estradiol and FSH are required only if patient is under 60 years of age and has not had a bilateral oophorectomy.

14. DRUG FORMULATION AND PROCUREMENT

Rapamune® (sirolimus) is an immunosuppressive agent. Sirolimus is a macrocyclic lactone produced by *Streptomyces hygroscopicus*. The chemical name of sirolimus (also known as rapamycin) is (3*S*,6*R*,7*E*,9*R*,10*R*,12*R*,14*S*,15*E*,17*E*,19*E*,21*S*,23*S*,26*R*,27*R*,34a*S*)-9,10,12,13,14,21,22,23,24,25,26,27,32, 33,34,34a-hexadecahydro-9,27-dihydroxy-3-[(1*R*)-2-[(1*S*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylethyl]-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3*H*-pyrido[2,1-c][1,4]

oxaazacyclohentriacontine-1,5,11,28,29(4*H*,6*H*,31*H*)-pentone. Its molecular formula is C51H79NO13 and its molecular weight is 914.2. The structural formula of sirolimus is shown below. The inactive ingredients in Rapamune® Tablets include sucrose, lactose, polyethylene glycol 8000, calcium sulfate, microcrystalline cellulose, pharmaceutical glaze, talc, titanium dioxide, magnesium stearate, povidone, poloxamer 188, polyethylene glycol 20,000, glyceryl monooleate, carnauba wax, *dl*-alpha tocopherol, and other ingredients. The 2 mg dosage strength also contains iron oxide yellow 10 and iron oxide brown 70.

14.1 Mechanism Of Action

Rapamycin inhibits T lymphocyte activation and proliferation that occurs in response to antigenic and cytokine (Interleukin [IL]-2, IL-4, and IL-15) stimulation by a mechanism that is distinct from that of other immunosuppressants. Rapamycin also inhibits antibody production. In cells, rapamycin binds to the immunophilin, FK Binding Protein-12 (FKBP-12), to generate an immunosuppressive complex. The rapamycin:FKBP-12 complex has no effect on calcineurin activity. This complex binds to and inhibits the activation of the mammalian Target Of Rapamycin (mTOR), a key regulatory kinase. This inhibition suppresses cytokine-driven T-cell proliferation, inhibiting the progression from the G1 to the S phase of the cell cycle. Studies in experimental models show that rapamycin prolongs allograft (kidney, heart, skin, islet, small bowel, pancreatico-duodenal, and bone marrow) survival in mice, rats, pigs, and/or primates. Rapamycin reverses acute rejection of heart and kidney allografts in rats and prolongs the graft survival in presensitized rats. In some studies, the immunosuppressive effect of rapamycin lasts up to 6 months after discontinuation of therapy. This tolerization effect is alloantigen specific. In rodent models of autoimmune disease, rapamycin suppresses immune-mediated events associated with systemic lupus erythematosus, collagen-induced arthritis, autoimmune type I diabetes, autoimmune myocarditis, experimental allergic encephalomyelitis, graft-versus-host disease, and autoimmune uveoretinitis.

14.2 PRECAUTIONS

14.2.1 General

Rapamune® is intended for oral administration only.

Lymphocele, a known surgical complication of renal transplantation, occurred significantly more often in a dose-related fashion in patients treated with Rapamune®. Appropriate operative measures should be considered to minimize this complication.

14.2.2 Lipids

The use of Rapamune® in renal transplant patients was associated with increased serum cholesterol and triglycerides that may require treatment. In Studies 1 and 2, in *de novo* renal transplant recipients who began the study with normal, fasting, total serum cholesterol (<200 mg/dL) or normal, fasting, total serum triglycerides (<200 mg/dL), there was an increased incidence of hypercholesterolemia (fasting serum cholesterol >240 mg/dL) or hypertriglyceridemia (fasting serum triglycerides >500 mg/dL), respectively, in patients receiving both Rapamune® 2 mg and Rapamune® 5 mg compared with azathioprine and placebo controls. Treatment of new-onset hypercholesterolemia with lipid-lowering agents was required in 42 - 52% of patients enrolled in the Rapamune® arms of Studies 1 and 2 compared with 16% of patients in the placebo arm and 22% of patients in the azathioprine arm.

In Study 4 (cyclosporine withdrawal study) during the prerandomization period, mean fasting serum cholesterol and triglyceride values rapidly increased, and peaked at 2 months with mean cholesterol values > 240 mg/dL and triglycerides > 250 mg/dL. After randomization mean cholesterol and triglyceride values remained higher in the cyclosporine withdrawal arm compared to the Rapamune® and cyclosporine combination. Renal transplant patients have a higher prevalence of clinically significant

hyperlipidemia. Accordingly, the risk/benefit should be carefully considered in patients with established hyperlipidemia before initiating an immunosuppressive regimen including Rapamune®.

Any patient who is administered Rapamune® should be monitored for hyperlipidemia using laboratory tests and if hyperlipidemia is detected, subsequent interventions such as diet, exercise, and lipid lowering agents, as outlined by the National Cholesterol Education Program guidelines, should be initiated. In clinical trials, the concomitant administration of Rapamune® and HMG-CoA reductase inhibitors and/or fibrates appeared to be well tolerated. During Rapamune® therapy with cyclosporine, patients administered an HMG-CoA reductase inhibitor and/or fibrate should be monitored for the possible development of rhabdomyolysis and other adverse effects as described in the respective labeling for these agents.

14.3 ADVERSE REACTIONS

Rapamune® Oral Solution: The incidence of adverse reactions was determined in two randomized, double-blind, multicenter controlled trials in which 499 renal transplant patients received Rapamune® Oral Solution 2 mg/day, 477 received Rapamune® Oral Solution 5 mg/day, 160 received azathioprine, and 124 received placebo. All patients were treated with cyclosporine and corticosteroids. Data (≥ 12 months post-transplant) presented in the table below show the adverse reactions that occurred in any treatment group with an incidence of ≥ 20%. Specific adverse reactions associated with the administration of Rapamune® (sirolimus) Oral Solution occurred at a significantly higher frequency than in the respective control group. For both Rapamune® Oral Solution 2 mg/day and 5 mg/day these include hypercholesterolemia, hyperlipemia, hypertension, and rash; for Rapamune® Oral Solution 2 mg/day acne; and for Rapamune® Oral Solution 5 mg/day anemia, arthralgia, diarrhea, hypokalemia, and thrombocytopenia. The elevations of triglycerides and cholesterol and decreases in platelets and hemoglobin occurred in a dose-related manner in patients receiving Rapamune®. Patients maintained on Rapamune® Oral Solution 5 mg/day, when compared with patients on Rapamune® Oral Solution 2 mg/day, demonstrated an increased incidence of the following adverse events: anemia, leukopenia, thrombocytopenia, hypokalemia, hyperlipemia, fever, and diarrhea. In general, adverse events related to the administration of Rapamune® were dependent on dose/concentration.

14.4 Storage

Rapamune® Oral Solution bottles should be stored protected from light and refrigerated at 2°C to 8°C (36°F to 46°F). Once the bottle is opened, the contents should be used within one month. If necessary, the patient may store the bottles at room temperatures up to 25°C (77°F) for a short period of time (e.g., not more than 15 days for the bottles). An amber syringe and cap are provided for dosing and the product may be kept in the syringe for a maximum of 24 hours at room temperatures up to 25°C (77°F) or refrigerated at 2°C to 8°C (36°F to 46°F). The syringe should be discarded after one use. After dilution, the preparation should be used immediately.

Rapamune® Oral Solution provided in bottles may develop a slight haze when refrigerated. If such a haze occurs allow the product to stand at room temperature and shake gently until the haze disappears. The presence of this haze does not affect the quality of the product. Rapamune® tablets should be stored at 20° to 25°C (USP Controlled Room Temperature) (68° to 77°F). Use cartons to protect blister cards and strips from light. Dispense in a tight, light-resistant container as defined in the USP.

15. STATISTICAL CONSIDERATIONS

15.1 Study Population

This study proposes recruitment of women with DCIS, LCIS, ALH or ADH cases. The population in the San Antonio area are mostly Hispanics (~55%) and Caucasian (~45%).

15.2 Statistical and Data Analysis

Availability of case and justification of sample size. According to our co-investigator and breast pathologist, the surgeons at our cancer center operated 24 cases of DCIS in 2013.

Ages	**# of patients at** UT Health San Antonio Mays Cancer Center
< 50	7
50-59	10
> 60	7

We consider about 60-70% enrollment in 5-year recruitment duration, we shall be able to recruit about 50-60 patients with menopausal status clinically confirmed. Tissue from this study will be compared to DCIS specimens from patients not treated with rapamycin. These specimens will be collected through the ongoing biorepository.

Statistically significant differences of the histopathological parameters and the molecular markers between the core biopsy and surgical specimens due to the rapamycin treatment will be tested by Mantel-Haenszel test for categorical data with scores ranging from 0 to 4 (or higher) as shown in Figure 2. To reach statistical power >70%, a patient group size of 24 or more is required for us to detect the difference of a score of 0.3 between untreated biopsy and treated surgical specimens with the P value targeted at 0.05 for one-sided Mantel-Haenszel test.

The **Mantel-Haenszel test** (between Rapamycin treatment & non-participant patients) will be set up as follows.

Test between treatment & non-participant patients

Study		**IHC score ≤ 2**	**IHC score > 2**	**Percentage**
UT Health San Antonio	Rapamycin	# of patients (a)	# of patients (b)	a/(a+b)
	No Rapamycin	# of patients (c)	# of patients (d)	c/(c+d)

Mantel-Haenszel test will allow us to extend IHC score to full range (instead of dichotomizing the IHC score), as well as other sites if more patients will be enrolled at different sites (we will not lump all patients together into one group, in case the underlying population demographic is a concern at different enrollment sites). Significance will be determined at p-value < 0.05 (Chi-square distributed with one degree of freedom). During the recruitment period, we will monitor the IHC scores different from two groups (treated/untreated) with simple *t* test on average IHC score (across all patients within each group) for each marker, in order to assess the IHC effectiveness, enrollment progress and patient demographic parameters and their association to final study outcomes.

For statistical test within either treatment or control arm, we will use **McNemar's test** to examine IHC score differences between biopsy and surgical tissues (at significance level of 0.05). The 2x2 table set up as follows.

Test within treatment or no treatment

	After treatment

		IHC score ≤ 2	IHC score > 2
Before treatment	**IHC score ≤ 2**	# of patients with lower IHC scores before & after	# of patients with higher IHC score after treatment
	IHC score > 2	# of patients with lower IHC score after treatment	# of patients with higher IHC scores before & after

Similar to between arm test, during the recruitment period, we will monitor the IHC scores different within each arm with simple paired-*t* test on average IHC score difference for each marker, in order to assess the IHC effectiveness, enrollment progress and patient demographic parameters and their association to final study outcomes.

16. ADVERSE EVENTS REPORTING

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Subjects must be carefully monitored for AEs. Adverse events should be assessed in terms of their seriousness, intensity, and relationship to the study drug. Adverse events are reported in a routine manner at scheduled times during a trial. Additionally, certain adverse events must be reported in an expedited manner to allow for optimal monitoring of patient safety and care.

All patients experiencing an adverse event, regardless of its relationship to study drug, will be monitored until:

1. the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline;
2. there is a satisfactory explanation other than the study drug for the changes observed; or
3. death occurs.

16.1 Definitions & Descriptions

16.1.1 Adverse event (AE)

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention, whether or not related to the intervention. A “drug-related toxicity" includes as events, any toxicity considered related, probably related, or possibly related to study drugs. Toxicities clearly not related to the drug, such as disease progression, environmental, unrelated trauma may not be considered “drug related toxicity”.

16.1.2 Severity of AEs

All adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. The CTCAE v. 4.03 is available at:
http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

If no CTCAE grading is available, the severity of an AE is graded as follows:

- Mild (grade 1): the event causes discomfort without disruption of normal daily activities.

- Moderate (grade 2): the event causes discomfort that affects normal daily activities.
- Severe (grade 3): the event makes the patient unable to perform normal daily activities or significantly affects his/her clinical status.
- Life-threatening (grade 4): the patient was at risk of death at the time of the event.
- Fatal (grade 5): the event caused death.

16.1.3 Serious Adverse events

All SAEs, regardless of attribution, occurring during the study or within 30 days of the last administration of study drug must be reported upon discovery or occurrence. Additional expedited or routine reporting may be required, depending on the nature of the SAE. A "serious" adverse event is defined in regulatory terminology as any untoward medical occurrence that:

1. Results in death. If death results from (progression of) the disease, the disease should be reported as the event (SAE) itself.
2. Is life-threatening. The patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
3. Requires in-patient hospitalization or prolongation of existing hospitalization for ≥ 24hours.
4. Results in persistent or significant disability or incapacity.
5. Is a congenital anomaly/birth defect.
6. Is an important medical event.
7. Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of "Serious Adverse Event". Examples: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.

16.1.4 Exceptions to the Definition of SAE

Certain hospitalizations or prolongation of hospitalizations should not be considered SAEs, including those that meeting the following criteria:

1. An admission resulting in a hospital stay of less than 24 hours.
2. An admission that is pre-planned (i.e. elective or scheduled surgery arranged prior to the start of the study).
3. An admission that is not associated with an AE (i.e. social hospitalization for purposes of respite care).

16.1.5 Unanticipated Problems Involving Risks to Subjects or Others (UPIRSO)

A UPIRSO includes events that meet ALL of the following criteria:

1. Are unanticipated in terms of nature, severity, or frequency;
2. Place the research subject or others at a different or greater risk of harm; AND
3. Are deemed to be related or possibly related to participation in the study.

16.2 Adverse event reporting

Steps to determine if expedited reporting is required include all events that occur within 30 days of the last dose of protocol treatment. Any event that occurs more than 30 days after the last dose of treatment

and is attributed (possibly, probably, or definitely) to the agent, must also be reported accordingly. You are required to:

1. **Identify** the type of adverse event using the NCI CTCAE v 4.03.
2. **Grade** the adverse event using the NCI CTCAE v 4.03.
3. **Determine** whether the adverse event is related to the protocol therapy.

Attribution categories are as follows:
Definite: AE is clearly related to the study treatment (attribution should be separated by each study drug)
Probable: AE is likely related to the study treatment (attribution should be separated by each study drug)
Possible: AE may be related to the study treatment (attribution should be separated by each study drug)
Unrelated: AE is clearly NOT related to the study treatment (attribution should be separated by each study drug

Determine the prior experience of the adverse event. Expected events are those that have been previously identified as resulting from administration of the specific agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in the current protocol and/or the drug package insert.

16.3 Expedited SAE Reporting

All SAEs must be reported following the DSMP outlined in *Appendix 2* of this protocol within 24 hours of becoming aware of the event. Completion of the UT Health San Antonio Mays Cancer Center SAE Form is required. The completed form should assess whether or not the event qualifies as a UPIRSO. The report should also include**:**

1. Protocol description and number
2. Patient's Identification Number
3. Description of event, severity, treatment, and outcome if known
4. Supportive laboratory results and diagnostics
5. Hospital Discharge Summary (if available)

All SAEs will be reported to, and reviewed by, the DSMB at their next meeting.

16.4 Reporting to the UT Health San Antonio IRB

Any death of a subject that is unanticipated in nature and at least possibly related to study participation will be promptly reported to the UT Health San Antonio Mays Cancer Center Regulatory Affairs Division for reporting within 24 hours of notification. Other SAE's that are life threatening, but not fatal, should be reported to UT Health San Antonio Mays Cancer Center Regulatory Affairs within 7 days of notification.

The following SAEs will be reported to the UT Health San Antonio Mays Cancer Center Regulatory Affairs for reporting to the UT Health San Antonio IRB at the time of continuing review:

1. All deaths of UT Health San Antonio Mays Cancer Center subjects that were not previously reported
2. All deaths of non-UT Health San Antonio subjects that are deemed to be unanticipated in nature and unrelated to participation
3. Other UPIRSO's
4. All other SAEs not previously reported to the UT Health San Antonio IRB as UPIRSOs

In addition, participating sites should follow local guidelines for reporting of SAEs to their IRB as required.

16.5 Reporting to the FDA

The UT Health San Antonio Mays Cancer Center Regulatory Affairs division will notify FDA within 7 calendar days of any SAE that is associated with study treatment, is unexpected, and is fatal or life-threatening. The UT Health San Antonio Mays Cancer Center Regulatory Affairs Division will notify the FDA within 15 calendar days of any SAE that is associated with the study treatment, unexpected, and serious but not fatal or life-threatening. This includes any previous SAEs that were not initially deemed reportable, but are later determined to meet the criteria for reporting by the DSMB. In these instances, an FDA Med Watch Form will be completed.

16.6 Routine Reporting

All other adverse events, such as those that are expected, or are unlikely or definitely not related to the study participation, are to be reported on the appropriate eCRF according to the time intervals noted in the Schedule of Events. Routine AEs will be reviewed by the assigned DSMB for the study in accordance with the Mays Cancer Center's Multi-site DSMP Procedures. These will be reviewed by the DSMB on an on-going basis. A summary of all these events will be reported annually to the UT Health San Antonio IRB as part of the continuing review process.

17. STUDY MANAGEMENT

17.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

17.2 Registration Procedures

Patients may not begin protocol treatment prior to registration. All patient registrations will be centralized through the Research/Nurse Coordinator of this study at the Institute for Drug Development at the Mays Cancer Center of the UT Health San Antonio. Please contact the assigned UT Health San Antonio Mays Cancer Center Research/Nurse Coordinator for questions regarding patient registration procedures.

17.3 Access to REDCAP

Enrolled study patients will be recorded in the web-based application REDCAP. Please note that a username and password is required to use this program and will be provided prior to training on the REDCAP system.

17.4 Patient Referrals to the Study

For potential patients please email the Nurse/Research Coordinator prior to consenting a patient to determine whether a slot is available on the study. In order for patient referrals to be processed efficiently, study teams are asked to inform the Nurse/Research Coordinator of any enrollment deadline date, if any.

Once a patient is referred for possible study enrollment, the treating physician will be asked to review, sign and date the study Eligibility Checklist as verification that the patient has met all inclusion/exclusion criteria. It will be the responsibility of the treating physician to issue informed consent to the patient.

17.5 Data Management and Monitoring

This study will be monitored as an Investigational Initiated study, which requires auditing after the first patient has completed 1 cycle of treatment at each site and at least an annual audit, as outlined in the Mays Cancer Center's DSMP. Please refer to *Appendix 2* for more specific details on the monitoring plan. Data submission requirements can be found in *Appendix 3*.

17.6 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

17.7 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval.

For any such emergency modification implemented, the coordinating site must be notified of the event within five business days of making the change.

17.8 Other Protocol Deviations

According to the IRB, a protocol deviation is any unplanned variance from an IRB approved protocol that:

1. Is generally noted or recognized after it occurs
2. Has no substantive effect on the risks to research participants
3. Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
4. Did not result from willful or knowing misconduct on the part of the investigator(s).

A protocol deviation is considered an instance of promptly reportable event if the occurrence:

1. Has harmed or increased the risk of harm to one or more research participants.
2. Has damaged the scientific integrity of the data collected for the study.
3. Results from willful or knowing misconduct on the part of the investigator(s).
4. Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

17.9 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required. The written amendment, and if required the amended consent form, must be sent to the IRB for approval prior to implementation.

17.10 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that the study investigator must retain all study documentation pertaining to the conduct of a clinical trial.

17.11 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records or de identified documents to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator at each site and will require his/her final signature to verify the accuracy of the data.

17.12 Publication Policy

All potential publications and/or data for potential publications (e.g. manuscripts, abstracts, posters, clinicaltrials.gov releases) must be approved in by the PI of this study prior to release. If the investigator's wish to obtain monitored data prior to this point (or prior to the point dictated by study design), the investigator must send a written request for data to the PI of this study that includes justification. If the request is approved, data will be provided no later than 4 weeks after this request approval. The investigators are expected to use only monitored, accurate and approved data in all publications. The investigators should submit a copy of the manuscript to the biostatistician to confirm that approved data are used appropriately. Once the biostatistician gives final approval, the manuscript may be submitted to external publishers.

17.13 Pathology Requirements

Biopsy proven disease by confirmation of biopsy specimen at the treating institution pathology department is required for enrollment to the study. Biopsy material will be reviewed by the pathology department at the treating institution.

18. SAMPLES FOR BIOMARKER EVALUATION

Fresh tissue will be collected from patients with DCIS, LCIS, ALH or ADH treated with rapamycin. Tissue will be processed for IHC staining of p16, COX2, and Ki-67 in the core biopsy following the procedures described by Kerlikowske et al (66). We will also do IHC for autophagy markers, Beclin1 and LC3, and TUNEL assay to determine how rapamycin treatment may alter autophagy and apoptosis in the DCIS lesions.

To determine the effect of short-term rapamycin treatment on histopathological biomarkers in DCIS. A pathologist will quantify histopathological parameters including lesion size, nuclear grade, and presence of necrosis in each patient's surgical specimens, as well as IHC for the markers including p16, COX2, and Ki-67.

Three specific parameters will be evaluated:

1. Luminal-to-basal epithelial ratio, which will examine the MaSC in both luminal and basal cells, and their ratio, in order to address whether this ratio will change during aging;
2. Basal and luminal stem/progenitor cell frequency, which will examine the percentage of cells that are capable forming spheres, and whether these frequencies will change during aging; and
3. 3D structure regeneration frequency in serial passages, which will examine the self-renewal/regeneration capacity of the stem and progenitor cells that are capable of forming spheres in suspension culture and in 3D-ECM culture, and can re-form the spheres when the sphere-forming cells are dissociated and allowed to regenerate spheres.

All fresh tissue specimens will be delivered to Dr LuZhe Sun's laboratory located at:

UT Health San Antonio
7703 Floyd Curl Drive, Mail Code 7762
San Antonio, TX 78229-3900
Phone: 210-567-5746

APPENDIX 1 – Common Toxicity Criteria for Adverse Events

Toxicity will be graded according to the NCI's Common Toxicity Criteria for Adverse Events (CTCAE) version 4.03. The CTCAE version 4.03 can be accessed at the following link:
http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

APPENDIX 2 – Data Safety & Monitoring Plan

a. Data and Safety Monitoring Oversight

A Data and Safety Monitoring Plan is required for all individual protocols conducted at UT Health San Antonio Mays Cancer Center. All protocols conducted at UT Health San Antonio Mays Cancer Center are covered under the auspices of the UT Health San Antonio Mays Cancer Center Institutional Data Safety Monitoring Plan (DSMP).

The UT Health San Antonio Mays Cancer Center Institutional DSMP global policies provide individual trials with:

1. institutional policies and procedures for institutional data safety and monitoring,
2. an institutional guide to follow,
3. monitoring of protocol accrual by the UT Health San Antonio Mays Cancer Center Protocol Review Committee,
4. review of study forms and orders by the Forms Committee,
5. tools for monitoring safety events,
6. independent monitoring and source data verification by the UT Health San Antonio Mays Cancer Center QA Monitor/Auditor
7. monitoring of UPIRSO's by the Director of Quality Assurance and DSMC,
8. determining level of risk (Priority of Audit Level Score – PALS),
9. oversight by the Data Safety Monitoring Committee (DSMC), and
10. Verification of protocol adherence via annual audit for all Investigator Initiated Studies by the UT Health San Antonio Mays Cancer Center Quality Assurance Division.

b. Monitoring Progress and Safety

Due to the risks associated with participation in this protocol, the UT Health San Antonio Mays Cancer Center DSMB #2 in conjunction with the Principal Investigator will perform assessment of adverse events, adverse event trends and treatment effects on this study. The UT Health San Antonio Mays Cancer Center DSMB #2 acts as an independent Data Safety Monitoring Board (DSMB) for IIS conducted at UT Health San Antonio Mays Cancer Center. The UT Health San Antonio Mays Cancer Center DSMB #2 will monitor data throughout the duration of a study to determine if continuation of the study is appropriate scientifically and ethically. An additional layer of review is provided by the UT Health San Antonio Mays Cancer Center Data Safety Monitoring Committee (DSMC) who will review DSMB quarterly reports. Monitoring will be conducted every 12 months from the initiation of the study until the last enrolled patient has had the end of study visit.

Baseline adverse events and serious adverse events will be captured using the UT Health San Antonio Mays Cancer Center Master Adverse Events Document (or proper AE tracking mechanism as per Clinical Trial Office policy) for each patient using CTCAE V. 4.03 for the grading and attribution of adverse events *(Appendix 1).*

Usage of the UT Health San Antonio Mays Cancer Center Master Adverse Events Document (or proper AE tracking mechanism as per Clinical Trial Office policy) centrally documents:

1. the event and grades the seriousness of the event,
2. if the event was a change from baseline,
3. the determination of the relationship between the event and study intervention,
4. if the event was part of the normal disease process, and

5. What actions were taken as a result of the event.

c. Safety Definitions

For this study, the following safety definitions will be applicable:

Adverse Event Definition: An adverse event (AE) is defined as any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research For this study, all adverse events will be documented starting with day 7 and ending 30 days after the last dose of study drug is received.

Serious Adverse Event Definition: A serious adverse event (SAE) is defined as any adverse event that:

1. results in death;
2. is life-threatening (places the subject at immediate risk of death from the event as it occurred);
3. results in inpatient hospitalization or prolongation of existing hospitalization;
4. results in a persistent or significant disability/incapacity;
5. results in a congenital anomaly/birth defect; or
6. based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition

Unanticipated Problems Involving Risks to Subjects or Others Definition: Unanticipated problem involving risk to subjects or others includes any incident, experience or outcome that meets all of the following criteria:

unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied (note: the unfounded classification of a serious adverse event as "anticipated" constitutes serious non-compliance); definitely related or probably related to participation in the research; and suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized

d. Reporting Requirements

For this study, the Master Adverse Events Documents collected on patients for this protocol will be reviewed by the PI on a monthly basis to determine if a serious safety problem has emerged that result in a change or early termination of a protocol such as:

1. dose modification,
2. suspending enrollment due to safety or efficacy, or
3. termination of the study due to a significant change in risks or benefits.

AE's and SAE events that occur during clinical trials with or without an Investigational New Drug (IND) application are mandatory reports submitted to FDA via **Medwatch FDA F3500A within 15 days for events that have at least a possible relationship with the drug.**

e. Assuring Compliance with Protocol and Data Accuracy

As with all studies conducted at UT Health San Antonio Mays Cancer Center, the PI has ultimate responsibility for ensuring protocol compliance, data accuracy/integrity and responding to recommendations that emanate from monitoring activities. The research site will be audited for accuracy at the end of the treatment cycle for the first patient enrolled. Protocol compliance, data accuracy and reporting of events is further ensured by an annual audit conducted by the Data Safety Officer, whose audit report is shared with the PI, the research team, and will be reviewed by the UT Health San Antonio Mays Cancer Center DSMB.

f. UT Health San Antonio Mays Cancer Center DSMB Membership

The UT Health San Antonio Mays Cancer Center has two DSMB's with a primary set of members specific to the histology of the study consisting of UT Health San Antonio faculty and staff. This Protocol is under the jurisdiction of DSMB#2 for Solid Tumor Studies.

As per NCI guidelines and to eliminate conflict of interest (financial, intellectual, professional, or regulatory in nature), the UT Health San Antonio Mays Cancer Center DSMB specific to this study will not treat patients on this protocol. Usage of the DSMB specific to the histology has been created to ensure that experts in that histology are represented on the DSMB assembled for this protocol, but may be expanded, at the PI's discretion, to include other members which may include:

1. experts in the fields of medicine and science that are applicable to the study (if not currently represented on the DSMB),
2. statistical experts,
3. lay representatives,
4. multidisciplinary representation, from relevant specialties including experts such as bioethicists, biostatisticians and basic scientists, and
5. others who can offer an unbiased assessment of the study progress.

Additional or alternate membership of in the DSMB is selected by the DSMC chair, in conjunction with the PI of this protocol.

g. UT Health San Antonio Mays Cancer Center DSMB Charter and Responsibilities

The UT Health San Antonio Mays Cancer Center DSMB will provide information on the membership composition, including qualifications and experience to both the UT Health San Antonio IRB and UT Health San Antonio Mays Cancer Center PRC for review. The UT Health San Antonio Mays Cancer Center DSMB for this study will act as an independent advisory board to the PI and will report its findings and recommendations to the PI, the UT Health San Antonio IRB and the UT Health San Antonio Mays Cancer Center DSMC. UT Health San Antonio Mays Cancer Center DSMB reports will utilize the Investigator Initiated Study Quarterly DSMB Report Form and meetings will occur on a monthly basis to review any updates from the prior meeting.

Once the protocol is activated, if not already established elsewhere in the protocol the UT Health San Antonio Mays Cancer Center DSMB will establish and provide:

1. procedures for maintaining confidentiality;
2. statistical procedures including monitoring guidelines, which will be used to monitor the identified primary, secondary, and safety outcome variables;
3. consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study;

4. plans for changing frequency of interim analysis as well as procedures for recommending protocol changes;
5. recommendation of dose escalation, MTD recommendation of early termination based on efficacy results;
6. recommendation of termination due to unfavorable benefit-to-risk or inability to answer study questions;
7. recommendation of continuation of ongoing studies;
8. recommend modification of sample sizes based on ongoing assessment of event rates; and
9. review of the final results and publications.

APPENDIX 3 – Data Collection & Submission

Study-specific instructions regarding the entry and submission of data using paper CRFs or eCRFs using an electronic data system (e.g. REDCap), will be provided at the time of training prior to study activation. UT Health San Antonio Mays Cancer Center compliance policies of the Cancer Therapy & Research Center at the UT Health San Antonio DSMP regarding data collection and submission will be strictly enforced.

a. Research Chart

In addition to the regular hospital chart, a separate patient research folder will be kept which includes the patient's signed, dated informed consent document.

Case Report Forms to be submitted within 5 days of patient's first dose of study treatment:

- Master Adverse Event Form (or proper AE tracking mechanism as per Clinical Trial Office policy): *Update to reflect the adverse events that are present at baseline.*

Case Report Forms to be submitted no later than 1 week after End of Treatment Visit:

- Master Adverse Events Form (or proper AE tracking mechanism as per Clinical Trial Office policy): *All AEs should be finalized within 30 days of last dose*

Case Report Forms to be completed during follow-up:

- Master Adverse Events Form (or proper AE tracking mechanism as per Clinical Trial Office policy): *Reflecting 3-month toxicity status*

APPENDIX 4 – List of prohibited strong CYP3A4 inducers and inhibitors

Category	Drug Name
Strong CYP3A Inhibitors	Boceprevir, Clarithromycin, Cobicistat, Conivaptan, Danoprevir, Grapefruit juice (citrus paradisi fruit juice, 240 mL TID), Elvitegravir, Idealisib, Indinavir, Itraconazole, Ketoconazole, LCL161, Lopinavir, Mibefradil, Nefazodone, Nelfinavir, Posaconazole, Ritonavir, Saquinavir, Telaprevir, Telithromycin, Tipranavir, Troleandomycin, Voriconazole
Strong CYP3A Inducers	Avasimibe1,2, Carbamazepine, Enzalutamide, Mitotane, Phenobarbital, Phenytoin, Rifabutin, Rifampin (Rifampicin)2, St. John's wort (hypericum perforatum)2

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