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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Triple-negative breast cancer (TNBC) is an overly aggressive breast cancer subtype that disproportionately affects African American women. Triple-negative breast cancer is characterized by a lack of expression of the estrogen and progesterone receptors as well as the absence of *HER2* amplification/mutation. *TP53* mutations are the only genetic correlate with poor clinical prognosis in this subtype. In our preliminary studies, we found that p53 mutations in TNBC often coincided with deletion/silencing of the *CDKN2A* locus that encodes both the ARF and INK4A tumor suppressors. Surprisingly, this genetic context was primarily present in African American women with TNBC. Concurrent loss of both p53 and *CDKN2A* function resulted in massive gains in proliferation and transformation of mouse and human mammary epithelial cells both in vitro and in vivo. These phenotypic tumor gains were the direct result of altered JAK1 (through loss of p53 and ARF) and CDK4 (through INK4A loss) activity. In this genetic context, JAK1 and CDK4 cooperate to stimulate breast tumor cell proliferation. ***For this reason, identifying these key growth-driving kinases is paramount to discovering novel combinatorial therapies for TNBC.*** In our year 2 report, we show that the JAK1 pathway is elevated in human TNBC that also have loss of ARF and p53 function. Moreover, we have determined that TNBC cell lines are uniquely sensitive to combined JAK1 and CDK4 inhibition in vitro.

15. SUBJECT TERMS
Breast cancer, ARF, INK4A, p53, metastasis, CDK4, mammary epithelial cells, patient-derived xenografts

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1. INTRODUCTION

Triple-negative breast cancer (TNBC) is an overly aggressive breast cancer subtype that disproportionately affects African American women. Triple-negative breast cancer is characterized by a lack of expression of the estrogen and progesterone receptors as well as the absence of *HER2* amplification/mutation. *TP53* mutations are the only genetic correlate with poor clinical prognosis in this subtype. In our preliminary studies, we found that p53 mutations in TNBC often coincided with deletion/silencing of the *CDKN2A* locus that encodes both the ARF and INK4A tumor suppressors. Surprisingly, this genetic context was primarily present in African American women with TNBC. Concurrent loss of both p53 and *CDKN2A* function resulted in massive gains in proliferation and transformation of mouse and human mammary epithelial cells both in vitro and in vivo. These phenotypic tumor gains were the direct result of altered JAK1 (through loss of p53 and ARF) and CDK4 (through INK4A loss) activity. In this genetic context, JAK1 and CDK4 cooperate to stimulate breast tumor cell proliferation. ***For this reason, identifying these key growth-driving kinases is paramount to discovering novel combinatorial therapies for TNBC.***

2. KEYWORDS

Breast cancer, ARF, INK4A, p53, metastasis, CDK4, mammary epithelial cells, patient-derived xenografts

3. ACCOMPLISHMENTS

Major Goals of the Project

There were three major goals for this first year of the grant proposal for Dr. Weber: 1) gain ACURO approval (Major Task 1, Subtask 1) and begin breeding the *TP53*$^{fl/fl}$/*CDKN2A*$^{fl/fl}$/K14-Cre and *TP53*$^{fl/fl}$/K14-Cre mice (Major Task 1, Subtask 2); 2) stain PDX (Major Task 2, Subtask 4) and human TNBC tumors (Major Task 2, Subtask 2 & 3) for each biomarker; and 3) treat cell lines with drugs and measure proliferation (Major Task 3, Subtask 2).

Goals Accomplished

We have now obtained ACURO approval (Major Task 1, Subtask 1), so we have been busty generating *TP53*$^{fl/fl}$/*CDKN2A*$^{fl/fl}$/K14-Cre and *TP53*$^{fl/fl}$/K14-Cre mice (Major Task 1, Subtask 2). We now have a colony of approximately 100 mice that we are actively monitoring for mammary tumor formation (Major Task 1, Subtask 2).

In Major Task 2, Subtask 4, we had proposed to analyze 30 PDX tumors for p53, ARF, INK4A, phospho-STAT1 and ISG15 proteins via immunohistochemistry. In year 1, we have stained and analyzed 15 PDX tumors for p53, ARF and ISG15. As shown in Table 1, we have now double-blinded scoring for ISG15, ADAR1, p14ARF and p53 in all human xenografts. The

WHIM	ISG15	ADAR1	P14	P53
2	2	3	0	1
3	3	3	0	1
4	3	1	2	1
5	3	1	1	0
6	3	1	2	1
10	1	2	0	1
12	2	2	0	2
13	1	2	0	3
14	1	2	0	3
17	2	1	1	2
21	1	2	2	3
25	2	1	1	3
29	2	1	2	2
30	3	2	1	1
31	2	3	1	3
34	2	3	1	3
36	1	3	0	3
41	2	3	2	3
46	2	2	1	1
48	2	3	1	2
50	2	2	1	1
52	2	2	1	1
53	0	1	1	2
54	2	1	1	0
55	1	0	1	1
65	2	2	1	0
68	1	1	2	1
69	3	1	1	0
71	2	2	2	2
Total Positive	21	18	9	14
Percent Postive	72.4138	62.069	31.0345	48.2759

Table 1. IHC of Human PDX Tumors

WashU Human In Mouse (WHIM) xenografts are numbered in the far left column. The relative staining for each protein is provided as a numerical score with '0' being no staining and '3' being the highest level of staining.

In Major Task 2, Subtasks 2 and 3, we had proposed to stain 525 primary TNBC samples and provide Allred scoring. In Figure 1, we now show a representative staining of 50 patient samples for ADAR1, p14, and p53 proteins.

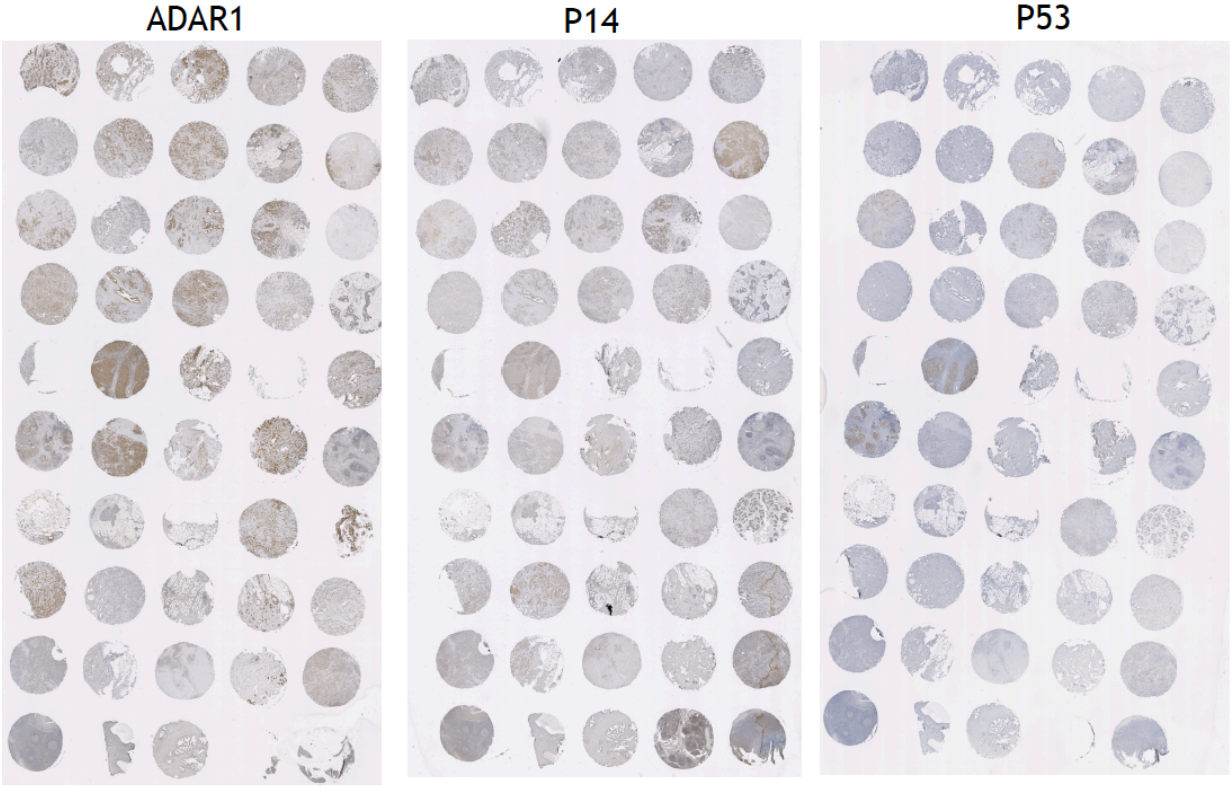

Figure 1. Human TNBC samples were used to generate a 525-core tissue microarray. Tissues were immunostained with antibodies recognizing human ADAR1, p14, and p53. H&E staining was also performed for each sample to provide adequate pathology reports.

We have now completed the staining of all 525 patient samples and have begun our final analysis to generate Allred scores for each sample and each protein (Major Task 2, Subtask 3).

In Major Task 3, Subtask 2, we proposed to determine the effects of CDK4 and JAK inhibitors on breast cancer cell line in vitro proliferation. To do this, we cultured three established human breast cancer cell lines that we received commercially from ATCC: HCC1806, SKBR3, and MDA-MB-231. Cells were initially cultured in complete media and then treated with the indicated amounts of drug. Proliferation was measured as an increase in total cell number by counting subsequent cells 48 hours after the initial treatment. Our results show that the two TNBC cell lines, HCC1806 and MDA-MB-231, are sensitive to both Ruxolitinib (JAK1 inhibitor) and Palbociclib (CDK4 inhibitor), while the HER2+ cell line, SKBR3, is largely resistant (Figure 2). Moreover, the proliferation of the HCC1806 TNBC cell line appears to be synergistically inhibited by the combination of Ruxolitinib and Palbociclib treatment (Figure 2). We will follow up

on this exciting data using additional TNBC cell lines and concentrations of drugs (Major Task 3, Subtask 2) as well as in vivo mouse experiments (Major Task 3, all Subtasks) in the final year.

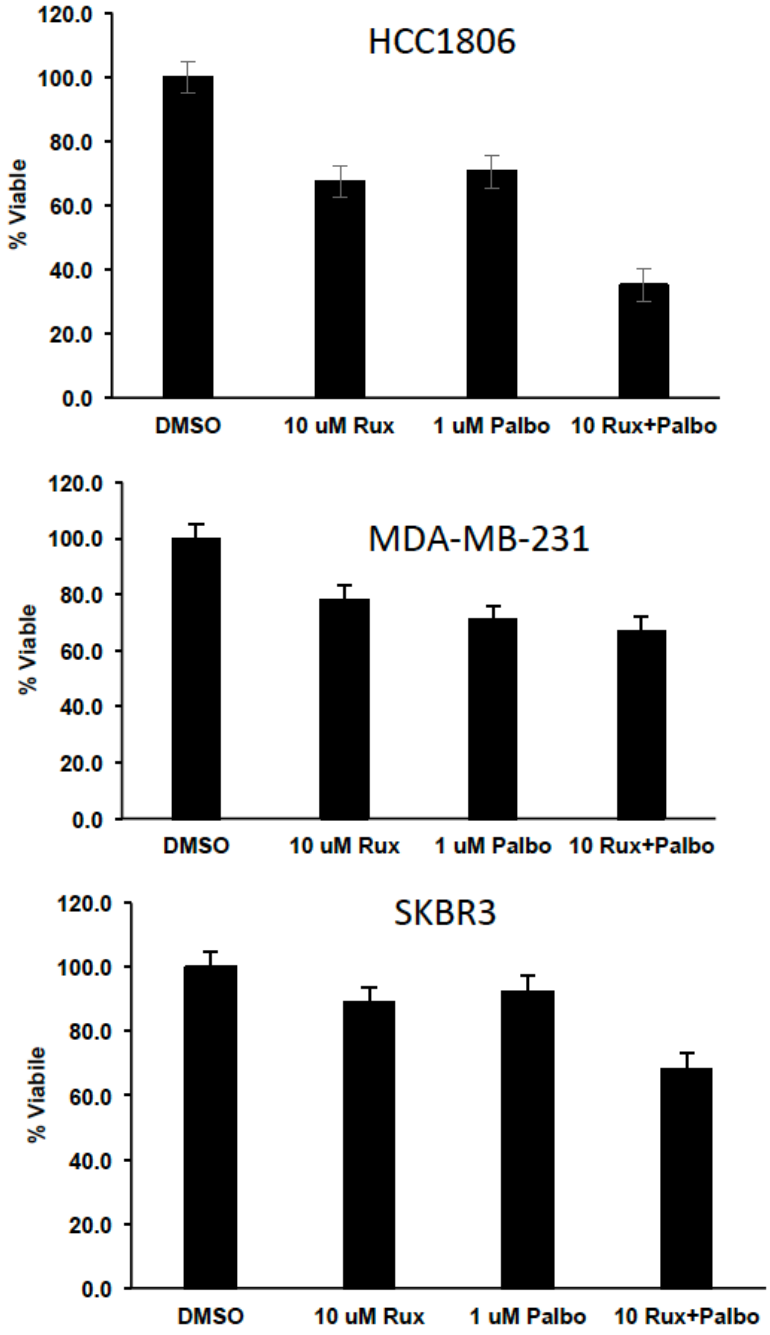

Figure 2. Treatment of breast cancer cell lines with CDK4 and JAK1 inhibitors. TNBC cell lines, HCC1806 and MDA-MB-231 and HER2+ SKBR3 cells were cultured and treated with indicated concentrations of Ruxolitinib (JAK1 inhibitor) and/or Palbociclib (CDK4 inhibitor) for 48 hours. Cells were then harvested and subjected to cell counting and Annexin V staining for viability.

Training Opportunities
Nothing to Report

Results Disseminated to the Community
I was able to present our findings to both the Dean of the medical school and his council as well as the Alumni Council of the medical school. We presented our initial findings at the San Antonio Breast Cancer Conference and the Cancer Biology Training Consortium in 2019.

Plans for Next Reporting Period
In the final year, we will focus our attention on the in vivo mouse models. Specifically, we will generate the *TP53*$^{fl/fl}$/*CDKN2A*$^{fl/fl}$/K14-Cre and *TP53*$^{fl/fl}$/K14-Cre mice (Major Task 1, Subtask 1). We will also focus on harvesting mammary tumors from these mice (Major Task 1, Subtask 2 & 3) as described in our first major task. We will also finish experiments aimed at determining the expression of this pathway using additional TNBC patient derived xenografts from Caucasians and African Americans (Major Task 2, Subtask 4). Finally, we will complete the in vivo treatment of de novo mouse tumors (Major Task 4, Subtask 1) and human PDX tumors (Major Task 4, Subtask 2) with CDK4 and JAK1 inhibitors followed by immunohistochemistry analysis of tissues (Major Task 4, Subtask 3).

4. IMPACT
Impact on Principal Discipline
Our current work will be incredibly impactful for those studying breast cancer aggression in vitro and in vivo. We have uncovered a novel pathway underlying the ability of breast cancer epithelial cells to proliferate at a high rate and readily form transformed colonies in soft agar. These are all hallmarks of aggressive tumors. In year 3, we will complete our studies by treating tumors in vivo with inhibitors of CDK4 and JAK1, hoping to underscore the importance of this pathway in tumor aggressiveness and metastasis.

Impact on Other Disciplines
Nothing to Report

Impact on Technology Transfer
Nothing to Report

Impact on Society
We have disseminated the data and ideals from this grant proposal to several groups in the St. Louis community. They were encouraged by our progress and excited about the future clinical impact our work might provide.

5. CHANGES/PROBLEMS
Changes in Approach
Nothing to Report

Anticipated Problems or Delays
Nothing to Report

Changes in Human, Animal, Biohazards and/or Selective Agents
Nothing to Report

6. PRODUCTS
Publications, Conference Papers and Presentations

The work was presented at the following conferences:

- Dean's Council, Washington University (Oral Presentation)
- Alumni Council, Washington University (Oral Presentation)
- San Antonio Breast Cancer Meeting (Poster Presentation)
- Cancer Biology Training Consortium, Baltimore, MD (Oral Presentation)

Internet Sites
Nothing to Report

Technologies or Techniques
Nothing to Report

Inventions, Patents and/or Licenses
Nothing to Report

7. PARTICIPANTS

Individuals That Have Worked on Project

Name:	Jason D. Weber
Project Role:	PI
Nearest person month worked:	1.2
Contribution to Project:	Dr. Weber served as the mentor for Dr. Kung in planning all experiments and overseeing the final data analysis.
Funding Support:	NIH R01CA190986

Name:	Shunqiang Li
Project Role:	Co-Investigator
Nearest person month worked:	0.6
Contribution to Project:	Dr. Li is growing all of the patient-derived tumors for both specific aims
Funding Support:	None

Name:	Pat Kung
Project Role:	Staff Scientist
Nearest person month worked:	12
Contribution to Project:	Dr. Kung was responsible for all of the mouse breeding (Task 1) in vitro assays in year 2 (Major Task 3) and the immunohistochemistry (Major Task 2).
Funding Support:	None

Changes in Active Other Support for PD/PI
Nothing to Report

Other Organizations Involved as Partners
Nothing to Report