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TITLE: Targeting Tumor-Intrinsic Immunosuppressive Mechanisms to Enhance Efficacy of Immune Checkpoint Blockade in Lung Cancer

PRINCIPAL INVESTIGATOR: Vivek Mittal, PhD

CONTRACTING ORGANIZATION: Joan & Sanford I. Weill Medical College of Cornell University

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Vivek Mittal, PhD

E-Mail: vim2010@med.cornell.edu

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Joan & Sanford I. Weill Medical
College of Cornell University
1300 York Avenue, Box 89
New York, NY 10065

8. PERFORMING ORGANIZATION REPORT NUMBER

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Relevance. This project addresses LCRP overarching challenges of understanding the molecular mechanisms of initiation and progression to clinically significant lung cancer, and identification of innovative strategies for prevention and treatment of lung cancer. Lung cancer is highly prevalent in both veterans and active-duty personnel due to exposures to mutagens in industrial substances, cigarette smoke, asbestos bearing materials, and battlefield air pollution. The cost of lung cancer to the VA has been suggested to be > $1 billion a year. This study will mechanistically dissect the IRE1α-XBP1 axis that constitutes a major immunosuppressive barrier that limits the efficacy of checkpoint blockade in NSCLC. Targeting the IRE1α has the immense potential to enhance the efficacy of PD-1 inhibition so that a larger cohort of NSCLC patients benefit.

Background. Mutant KRAS represents >30% NSCLC, and currently possess no effective therapeutic options. *KRAS* mutations typically predict a lack of response to conventional therapies and therefore, treatment of *KRAS* adenocarcinomas is an urgent unmet clinical need. We posit that targeting the ER stress IRE1α-XBP1 pathway has potential in the treatment of high-risk NSCLC patients.

Overarching challenges. Of the 1.8 million individuals diagnosed per year worldwide, approximately 1.6 million succumb to death. Non-small cell lung cancer (NSCLC) constitutes 85-90% of all lung cancer. KRAS is the most frequently occurring oncogenic mutation in NSCLC, representing ~20-30% of NSCLC. Moreover, KRAS mutations are associated with a poor prognosis, and reduced benefit from adjuvant chemotherapy, compared with the general NSCLC population. Despite this clinical significance, there is not a single effective FDA approved targeted therapy against KRAS. Given the unmet clinical need, there is an urgent requirement to develop targeted therapeutic approaches for effective treatment of mutant KRAS NSCLC.

Hypothesis /Objective. We hypothesize that interventions against IRE1α-XBP1 signaling either alone or in combination with immune checkpoint blockade can be developed into a viable therapeutic strategy for currently untreatable KRAS mutant patients. Using a combination of genetic and pharmacological approaches, we propose to achieve the following objectives: *1*) dissect the mechanisms by which cancer intrinsic IRE1α-XBP1 signaling generates immunosuppressive microenvironments in NSCLC, *2)* to assess whether pharmacological inhibition of IRE1α endoribonuclease can be used to target tumor progression, and *3*) to determine if pharmacological inhibition of IRE1α can used as a novel approach to enhance the effectiveness of immune checkpoint blockade in NSCLC. Our overall goal is to develop a mechanism-guided intervention against KRAS driven lung cancer.

Specific Aims. Using two independent but integrated aims, we propose*; 1*) To determine the mechanisms by which cancer cell-intrinsic loss of IRE1α-XBP1 signaling modulates anti-tumor immunity, and *2)* To evaluate the therapeutic potential of targeting the IRE1α-XBP1 pathway alone or in combination with PD-1 inhibition in NSCLC.

Study Design. Using a combination of genetic and pharmacological approaches, we propose to dissect the mechanisms by which IRE1α-XBP1 signaling in the tumor cells may elicit concomitant antitumor immunity in the tumor microenvironment through its role in activating tumor infiltrating lymphocytes, and simultaneously limiting immunosuppressive Tregs and MDSCs. Furthermore, the therapeutic potential of targeting the IRE1α-XBP1 axis in enhancing the efficacy of PD-1 blockade will be determined.

Innovation. This proposal is conceptually and technically innovative as it seeks to assess specific and direct inhibition of the IRE1α-XBP1 signaling pathway that has remained unexplored in lung cancer. A major conceptual innovation is that this study emphasizes that targeting cancer intrinsic IRE1α-XBP1 signaling has the potential to elicit concomitant antitumor immunity through its role in immune cell reprogramming. A variety of mouse genetic models, together with compartment-specific gene knockout strategies will be employed. In parallel, IRE1α inhibitors will be used to complement the genetic findings and enhance feasibility of clinical translation, and combined inhibition of IRE1α and PD-1 will be evaluated.

Impact. We expect that the mechanistic insights from the preclinical investigations will generate unique translational opportunities that may lead to the design of future clinical trials. The demonstration that targeting the IRE1α-XBP1 pathway may act synergistically with immune checkpoint blockade will allow future clinical trials to evaluate this new combination regimen in currently untreatable mutant KRAS lung cancer patients. Additionally, the XBP1 gene signature identified in human NSCLC has the potential to serve as a prognostic/diagnostic biomarker of the disease, and may allow monitoring efficacy of therapies targeting the IRE1α endoribonuclease.

15. SUBJECT TERMS XBP1, IREalpha, lung cancer, ER stress, immune checkpoint blockade, KRAS, immunosuppressive, T-cell, endoplasmic reticulum, immunophenotype, CRISPR					
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

This proposal aims to dissect the mechanisms by which cancer cell specific IRE1α-XBP1 signaling generates an immunosuppressive microenvironment that promotes tumor progression by inactivating cytotoxic T lymphocytes and simultaneously increasing immunosuppressive Tregs and MDSCs. Overcoming immunosuppression in the tumor microenvironment is a fundamental pre-requisite for the success of clinically relevant immune checkpoint inhibitors including anti-PD-1 and CTLA4. We posit that treatment with IRE1α selective small molecule inhibitors will overcome major immunosuppressive barriers and boost anti-tumor immunity in NSCLC, which in turn may constitute a new approach in enhancing the efficacy of immune checkpoint inhibitors in NSCLC. This approach has the potential to increase the current objective response rates 17-20% with immune checkpoint inhibitors in NSCLC. We expect that the mechanistic insights from these investigations will generate unique translational opportunities that may lead to the design of future clinical trials for currently untreatable mutant KRAS patients.

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

XBP1, IRE1α, lung cancer, ER stress, immune checkpoint blockade, KRAS, immunosuppressive, T-cell, endoplasmic reticulum, immunophenotype, CRISPR

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.

Aim 1: Determine the mechanisms by which cancer cell- intrinsic loss of IRE1α-XBP1 signaling modulates anti-tumor immunity (1-24 months)

Subaim 1.1 Identify IRE1α/XBP1regulated immunomodulators. (1-6 months)

Task: Perform integrated pathway and gene set enrichment in order to identify known and novel candidate immunomodulators to be evaluated (1-6 months) **(See Fig. 1)**

Subaim 1.2: IRE1α-XBP1 mediated gene signatures via RNA-seq in human NSCLC. (4-18 months)

• identify differentially regulated genes in tumor epithelial cells compared to epithelial cells from matched adjacent lungs **((See Fig. 2-3)**

• integrated cross-species analysis will be performed at the gene (focusing on orthologs) and pathway level to confirm HKP1 findings

Task: Use prospectively collected surgically resected fresh specimens to sort tumor epithelial cells from 20 KRAS patients

Subaim 1.3: Dissect the IRE1α-XBP1-PGE2 axis. (4-24 months)

Task 1.3.1: Evaluate XBP1s-mediated transcriptional regulation of Ptges and Ptgs2 (COX-2). Conduct (ChIP-qPCR) experiments to determine binding of XBP1s to the promoter of Ptges and Ptgs2 genes (samples from subaim 1.2). Validate the patient relevance of the XBP1s binding in vivo.

Task 1.3.2: Investigate the consequences of modulated PGE2 levels on the immune landscape of IRE1αKO HKP1 tumors. **(See Fig. 4)**
Collect BALF from IRE1αWT and IRE1αKO HKP1 tumors and perform immunophenotyping on the matched sets of lungs.
Control (n=10 mice) + Tumor bearing (n=10 mice) X 3 cell lines = 40 mice X 2 experiments= 80 BL6 mice

Task 1.3.3: Inducible expression of Ptges (mPGES1) in IRE1αKO HKP1 cells to rescue phenotype. Infect IRE1αWT and IRE1αKO cells with an inducible lentiviral Ptges1 cDNA.
Confirm the expression via qPCR for Ptges and ELISA for PGE2. Inject mice with Ptges+IRE1α WT or KO cell line. Evaluate in vivo tumor kinetics by BLI: survival experiment, immunophenotyping via flow cytometry Control (n=10 mice) + Tumor bearing (n=10 mice) X 3 cell lines = 40 mice X 2 experiments= 80 BL6 mice

Aim 1 Milestone(s) Achieved: Identify cancer cell intrinsic immunomodulators regulated by IRE1a-XBP1 signaling and evaluate the functional role of these modulators in reprogramming immune cells (6-24 months)

Local IRB/IACUC Approval (3 months)

Milestone Achieved: HRPO/ACURO Approval (4 months)

Aim 2: Evaluate the therapeutic potential of targeting the IRE1α-XBP1 pathway alone or in combination with PD-1 inhibition in NSCLC (6-24 months)

Subaim 2.1: Evaluate pharmacological inhibition of IRE1α or XBP1 as a therapeutic strategy in NSCLC. (3-18 months) **(See Fig. 5-6)**

Task: Determine the efficacy of single agent IRE1α targeting drugs. Administer B-I09 at 50mg/kg, 5 days a week for 3 weeks. Cohorts of KO and WT mice will be challenged with HKP1or CMT 167 tumor cells (n=10 per group). Tumor nodules, proliferation (Ki-67), apoptosis (cleaved Caspase 3) and microvessel density will be evaluated (IF staining, FACS-based immunophenotyping).
Control (n=10 mice) BL6 mice + Tumor bearing (n=10 mice) X 3 specific KOs = 40 mice X 2 experiments= 80 Bl6 mice

Subaim 2.2: Determine if targeting the IRE1α -XBP1 axis acts synergistically with PD-1 inhibition. (12-24 months)

Task: Mice bearing HKP1 lung adenocarcinoma (n=10 per group) will be administered vehicle or IgG antibody (control), B- I09 (50 mg/kg), anti-PD1 antibody (clone RMP1-14 from BioXCell, 250 ug/mouse twice a week for two weeks) or B-I09 plus anti-PD1 antibodies.
Control (n=10 mice) BL6 mice + Tumor bearing (n=10 mice) X 5 treatments x 2 dosages = 100 mice X 2 experiments= 200 Bl6 mice

Determine: i) the number and proportion of activated, antigen-experienced T cells infiltrating lung tumors, ii) the capacity of tumor-infiltrating T cells to effectively respond to tumor antigens, iii) the number and proportion of tumor-specific T cells exhibiting central memory-like markers (CD62L+CD44+), in lymphoid tissue and bone marrow, and iv) the number and proportion of tumor-infiltrating Treg cells in NSCLC tumors and associated lymphoid organs.

Memory T cells isolated from treated tumor-bearing mice will be adoptively transferred into different groups of naive mice, and animals will be challenged 24 hours later with wild-type HKP1 cells. Tumor growth will be analyzed
Total mice = 100 Bl6 mice

Milestone(s) Achieved: 1) Treatment with IRE1α selective small molecule inhibitors will boost anti-tumor immunity and impair tumor progression in NSCLC; 2) determine whether targeting IRE1α catalytic function has the potential in enhancing the efficacy of PD-1 blockade in NSCLC (12-24 months)

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Aim 1: Determine the mechanisms by which cancer cell- intrinsic loss of IRE1α-XBP1 signaling modulates anti-tumor immunity.

Subaim 1.1 Identify IRE1α/XBP1regulated immunomodulators.
Task: Perform integrated pathway and gene set enrichment in order to identify known and novel candidate immunomodulators to be evaluated

Progress: To identify IRE1α -XBP1-regulated genes, we used our previously generated RNA-seq data from mCherry+ tumor epithelial cells from IRE1α^{WT} or IRE1α^{KO} tumors at two different time points of growth: day 10, when tumor growth is comparable, and day 14, when tumors show regression phenotypes as a consequence of IRE1α loss. We performed ingenuity pathway analysis (IPA) of differentially regulated genes in IRE1α^{KO} vs IRE1α^{WT} tumor cells identified key upstream regulators implicated in induction of type-I IFN signaling and suppression of PTGR4 (receptor of PGE2) signaling **(Fig. 1).** We posit PGE2 and type-I IFN signaling, as a mechanism dominantly exploited by lung cancer cells to mediate crosstalk with the immune to cells to drive immunosuppression and promote malignant progression in NSCLC.

Upstream Regulator	Predicted Activation State	Activation z-score	p-value of overlap
IFNG	Activated	8.492	8.71E-49
TNF	Activated	7.78	2.18E-28
MYD88	Activated	7.661	9.52E-20
IRF3	Activated	7.556	5.46E-31
TLR4	Activated	7.147	4.57E-27
TICAM1	Activated	7.041	9.51E-30
Ifnar	Activated	6.843	1.71E-27
STAT1	Activated	6.743	4.69E-38
IRF7	Activated	6.333	7.76E-21
SAMSN1	Activated	6.172	3.47E-16
MAP2K5	Inhibited	-4.317	6.99E-11
RNF31	Inhibited	-4.491	1.20E-12
IL1RN	Inhibited	-4.799	3.29E-08
Nr1h	Inhibited	-4.91	1.34E-15
PTGER4	Inhibited	-4.978	1.15E-33
SOCS1	Inhibited	-5.017	3.60E-20
GFI1	Inhibited	-5.346	1.02E-11
ACKR2	Inhibited	-5.354	5.03E-17
TRIM24	Inhibited	-5.684	1.09E-19
PSMB11	Inhibited	-5.719	1.37E-17
PNPT1	Inhibited	-5.724	2.87E-23

Fig. 1. Upstream regulator analysis. A. Differentially expressed genes between IRE1a^{KO} and IRE1a^{WT} mCherry+ cancer cells analyzed by Ingenuity Pathway Analysis (Qiagen) for upstream regulators. Highlighted are upregulated (Red) and downregulated (blue) candidates with p values. Purple dots represent factors implicated in type-I IFN signaling. Orange dot indicates the PGE_2 receptor EP4

Subaim 1.2: IRE1α-XBP1 mediated gene signatures via RNA-seq in human NSCLC.
Task. Use prospectively collected clinical specimens to sort tumor epithelial cells and perform RNA-seq analysis to identify XBP1 gene signatures.

Progress: We have taken a slightly different approach to identify IRE1a gene signature that can predict survival in NSCLC patients (**Fig. 2A**). We used the differentially regulated genes derived from RNA-seq data of HKP1 cells from IRE1α^{WT} and IRE1α^{KO} tumors. To determine if these alterations can be developed as gene signatures of prognostic value in human NSCLC, we applied various cutoffs including fold change (FC) and false discovery rate (FDR) to identify an optimal gene signature associated with survival in human NSCLC. We then applied these gene signatures for unsupervised clustering of a discovery cohort of >300 LUADs available from the TCGA database and performed single sample gene set enrichment analysis (ssGSEA). Samples stratified into the top and bottom

tertiles for each signature were evaluated for survival. Of the various cut-offs applied, we selected log2FC>1 and FDR 1% gene signature, as patients enriched for genes upregulated in the IRE1α^{KO} murine tumor cells (IRE1α^{KO} high comprised of 582 genes) demonstrated enhanced survival, whereas patients enriched for downregulated gene signature (IRE1α^{KO} low comprised of 144 genes) showed decreased survival (**Fig. 2B).**

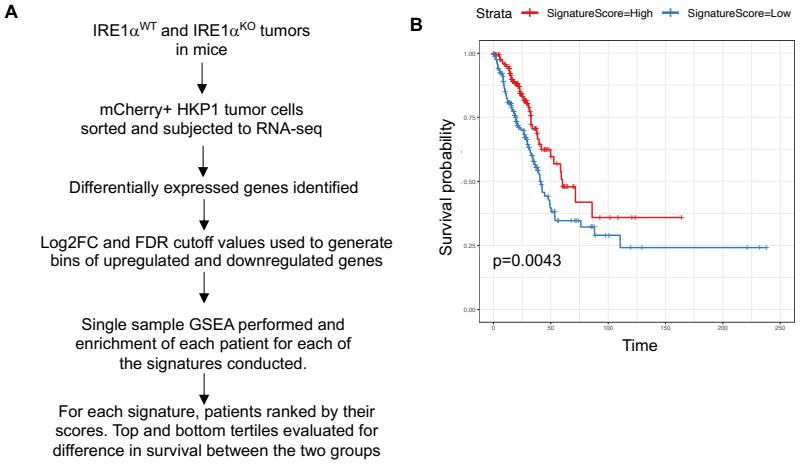

Fig. 2. **(A)** Schematic showing discovery of murine IRE1a gene signature from RNA-seq data. **(B)** IRE1a KO signature for genes upregulated in the murine IRE1aKO cells and downregulated in the WT cells (Log2 Fold change>1 and FDR 0.01) applied to the TCGA LUAD dataset and each patient's enrichment for the genes was evaluated by using single sample GSEA (ssGSEA). These patients were then stratified into the top and bottom tertiles for each signature and evaluated for survival. n=169 patients each.

To determine if the IRE1α^{KO} mediated immune alterations in mouse tumors are relevant to human NSCLC, we used the xCell pipeline to calculate an immune score and to enumerate different cell types in the TME. Specifically, xCell was used to deconvolute differentially expressed genes in IRE1α^{KO} high and low signature patients from the top and bottom tertiles from the TCGA LUAD samples. Using the immune enrichment scoring, we observed that patients enriched for the IRE1α^{KO} high signature, demonstrated increased deconvolution-based immunoscore (**Fig. 3A**). In addition, an increase in cDC and CD8+ T cells (**Fig. 3B-C**) was consistent with the observation in the mouse model. Unexpectedly, Tregs appeared to be increased (**Fig. 3D**).

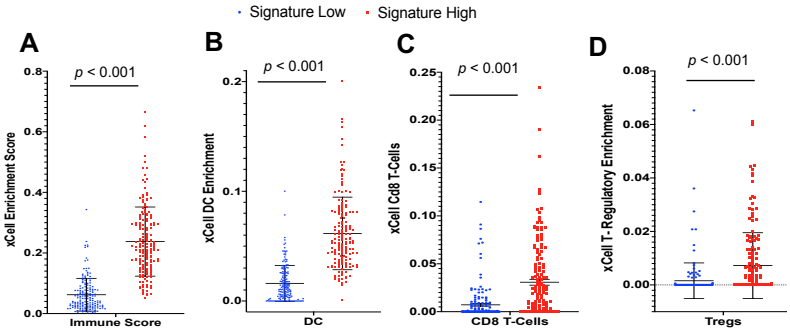

Fig. 3. **Immune cell deconvolution.** Patients from the IRE1a^{KO} signature (low and high) analyzed through the xCell deconvolution pipeline to determine compositions of various immune cells. Immune score, DCs, CD8+ T cells and T-regs were elevated patients enriched for the murine IRE1a^{KO} signature upregulated genes. Blue, signature low, red, signature high.

Subaim 1.3: Dissect the IRE1α-XBP1-PGE2 axis.

Task 1.3.3: Inducible expression of Ptges (mPGES1) in IRE1αKO HKP1 cells to rescue phenotype. Infect IRE1α^{WT} or IRE1α^{KO} cells with an inducible lentiviral Ptges1 cDNA.

Progress: To directly establish the direct role of IRE1α-XBP1-PGE$_2$ axis in malignant progression, we have generated IRE1α^{WT} and IRE1α^{KO} HKP1 cells stably expressing the Ptges1 cDNA (**Fig. 4A**). In addition, we also generated IRE1α^{WT} and IRE1α^{KO} HKP1 cells stably expressing the XBP1s cDNA (**Fig. 4B**), as this will be useful to determine if the impact of IRE1a is directly through the IRE1α-XBP1 axis.

Aim 2: Evaluate the therapeutic potential of targeting the IRE1α-XBP1 pathway alone or in combination with PD-1 inhibition in NSCLC

Subaim 2.1: Evaluate pharmacological inhibition of IRE1α or XBP1 as a therapeutic strategy in NSCLC.

Progress: In our proposal, we had indicated that we will use B-I09, a selective and potent small molecule inhibitor of the IRE1α RNAse activity. However, we have decided to use another inhibitor MKC8866, a potent and selective inhibitor of IRE1α kinase domain[42]. We chose MKC8866 because of its favorable reported pharmacokinetics and safe toxicity profiles in vivo. Treatment of HKP1 cells with MKC8866 showed marked reduction of XBP1s, while XBP1t remained unchanged **(Fig. 5)**

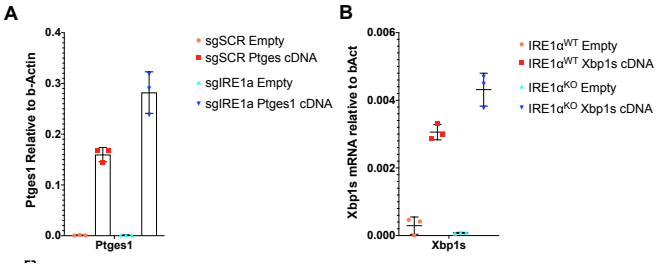

Fig. 4. Rescue of IRE1α^{KO} phenotypes with ectopic expression of Ptges1 and XBP1s. (A). Stable expression of Ptges1 cDNAs in IRE1α^{WT} or IRE1α^{KO} HKP1 cells evaluated by qPCR. **(B).** Stable expression of XBP1s cDNAs in IRE1α^{WT} or IRE1α^{KO} HKP1 cells evaluated by qPCR.

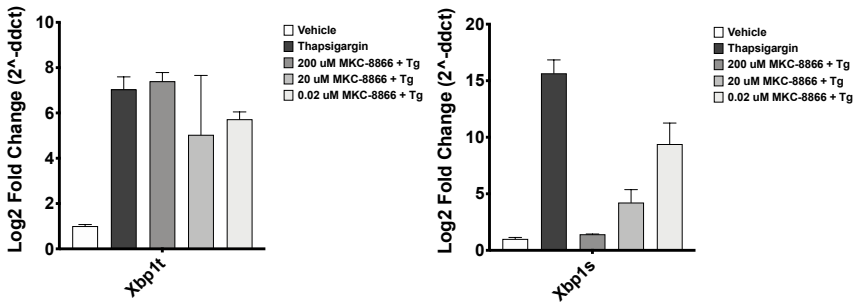

Fig. 5. Treatment of HKP1 cells with vehicle (control) and various concentrations of MKC-8866. Thapsigargin was used to induce ER stress.
Q-PCR for XBP1 total (XBP1t) and XBP1 spliced (XBP1s) are shown in the left and right panels respectively.

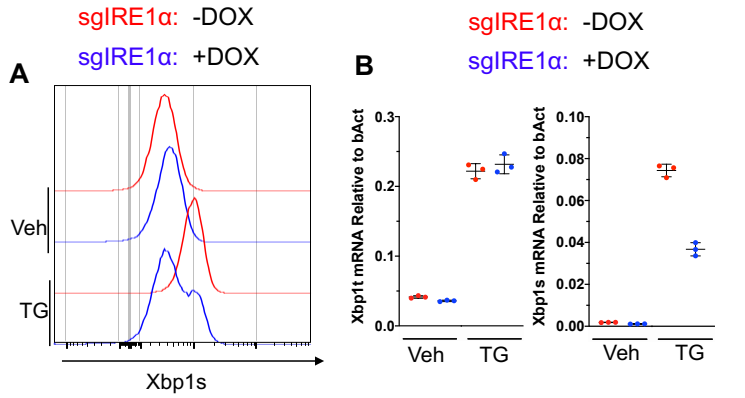

Fig. 6. Doxycyclin inducible CRISPR/Cas9 mediated knockdown of IRE1a in HKP1 cells. Cas9-Dox inducible HKP1 cells were treated with Vehicle or Doxycycline for 3 days. These cells were then treated for 6 hours with 1uM Thapsigargin (TG). Cells were then evaluated for XBP1s activity via flow cytometry with an anti-XBP1s antibody (BD Biosciences, 562821, Clone Q3-695) **(A),** or with qPCR for total Xbp1 **(B)** or Xbp1s **(C).**

To complement the pharmacological studies, we have also generated HKP1 c ells stably expressing doxycycline inducible knockout of IRE1α (**Fig. 6**). HKP1 cells stably expressing doxycycline inducible CRISPR/Cas9 showed marked reduction of XBP1 following treatment with dox as determined by flow cytometry analysis using a XBP1s specific antibody **(Fig. 6A).**
Consistent with flow cytometry, Q-PCR analysis showed a decrease in XBP1s following treatment with dox **(Fig. 6B).**

These cells will be used to determine if conditional loss of IRE1α impacts tumor growth, it will also be used to determine if IRE1a loss increases the efficacy of PD-1 blockade.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

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

In year 2, we will perform the following:

1) Ectopic expression of Ptges and XBP1 in IRE1a KO HKP1 tumors to rescue phenotypes in vivo.
2) Investigate the consequences of modulated PGE_2 levels on the immune landscape of $IRE1\alpha^{KO}$ HKP1 tumors.
3) Use IRE1a inhibitor monotherapy or in combination with checkpoint blockade to determine impact on HKP1 tumor progression

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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report for this reporting period.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report this period.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report for this reporting period.

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:*

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

The COVID-19 pandemic caused the partial shutdown of the laboratory from March 2020 – June 2020. This resulted in significant delay with experiments.

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.

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

N/A

Significant changes in use of biohazards and/or select agents

N/A

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**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

  Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report

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".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Vivek Mittal, PhD
Project Role: PI
Months worked: 1.2
Contribution to project: Dr. Mittal oversees all aspects of the proposal as PD/PI.

Name: Juan Cubillos-Ruiz, PhD
Project Role: Co-Investigator

Months worked: 0.6
Contribution to project: Dr. Cubillos-Ruiz collaborators with Dr. Mittal to investigate XBP1 regulated gene signatures

Michael Crowley, MS
Project Role: Graduate Student
Months worked: 6
Contribution to project: Mr. Crowley performed all in vitro work and data analysis.

Name: Sharrell Lee, MS
Project Role: Technician
Months worked: 4
Contribution to project: Ms. Lee assisted Mr. Crowley on all experiments.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Dr. Mittal received a two-year research grant UG3CA244697 from the NIH NCI entitled, "Intercepting progression from pre-invasive to invasive lung adenocarcinoma." Dates are from 09/25/2019 – 08/31/2021. He is spending 10% effort (1.2 calendar months).

Dr. Mittal's completed his grant W81XWH-16-1-0065 from the US DoD BCRP entitled, "Identification and Targeting of Metastasis Suppressing miRNAs in Triple Negative Breast Cancer." Dates were from 04/15/2016 – 04/14/2020.

Dr. Mittal completed the Metastasis Research Grant from the Free to Breathe Foundation (LCRF) on which he was a coPI along with Dr. Stiles and Dr. McGraw, entitled "ART1: a novel therapeutic target for the prevention of metastases from non-small cell lung cancer." Dates were 07/02/2015 – 12/31/2019

Dr. Mittal completed his Research Grant from the Metavivor Foundation entitled, "Targeting epigenetic regulator PRC2 as a therapy for established metastasis." Dates were 03/27/2018 – 03/26/2020.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*

- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site)*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *N/A*

QUAD CHARTS: *N/A*

9. APPENDICES: *N/A*