

AWARD NUMBER: W81XWH-19-1-0286

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

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REPORT DATE: AUGUST 2021

TYPE OF REPORT: Annual Technical Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE*Form Approved*
OMB No. 0704-0188

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1. REPORT DATE AUGUST 2021	2. REPORT TYPE Annual Technical	3. DATES COVERED 08/01/2020 - 07/31/2021
4. TITLE AND SUBTITLE Targeting Tumor-Intrinsic Immunosuppressive Mechanisms to Enhance Efficacy of Immune Checkpoint Blockade in Lung Cancer		5a. CONTRACT NUMBER W81XWH-19-1-0286
		5b. GRANT NUMBER
		5c. PROGRAM ELEMENT NUMBER
		5d. PROJECT NUMBER
6. AUTHOR(S) Vivek Mittal, PhD E-Mail: vim2010@med.cornell.edu		5e. TASK NUMBER
		5f. WORK UNIT NUMBER
		8. PERFORMING ORGANIZATION REPORT NUMBER
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		10. SPONSOR/MONITOR'S ACRONYM(S)
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012		
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited		
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 IRE1 α -XBP1 axis that constitutes a major immunosuppressive barrier that limits the efficacy of checkpoint blockade in NSCLC. Targeting the IRE1 α 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 IRE1 α -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 IRE1 α -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 IRE1 α -XBP1 signaling generates immunosuppressive microenvironments in NSCLC, 2) to assess whether pharmacological inhibition of IRE1 α endoribonuclease can be used to target tumor progression, and 3) to determine if pharmacological inhibition of IRE1 α can be 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 IRE1 α -XBP1 signaling modulates anti-tumor immunity, and 2) To evaluate the therapeutic potential of targeting the IRE1 α -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 IRE1 α -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 IRE1 α -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 IRE1 α -XBP1 signaling pathway that has remained unexplored in lung cancer. A major conceptual innovation is that this study emphasizes that targeting cancer intrinsic IRE1 α -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, IRE1 α inhibitors will be used to complement the genetic findings and enhance feasibility of clinical translation, and combined inhibition of IRE1 α 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 IRE1 α -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 IRE1 α endoribonuclease.

15. SUBJECT TERMS

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

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

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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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 IRE1 α -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 prerequisite for the success of clinically relevant immune checkpoint inhibitors including anti-PD-1 and CTLA4. We posit that treatment with IRE1 α 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, IRE1 α , 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 IRE1 α -XBP1 signaling modulates anti-tumor immunity (1-24 months)

Subaim 1.1 Identify IRE1 α /XBP1 regulated 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: IRE1 α -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 IRE1 α -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 IRE1 α KO HKP1 tumors. (See Fig. 4)

Collect BALF from IRE1 α WT and IRE1 α KO 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 IRE1 α KO HKP1 cells to rescue phenotype. Infect IRE1 α WT and IRE1 α KO cells with an inducible lentiviral Ptges1 cDNA.

Confirm the expression via qPCR for Ptges and ELISA for PGE2. Inject mice with Ptges+IRE1 α 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 IRE1 α -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 IRE1 α -XBP1 pathway alone or in combination with PD-1 inhibition in NSCLC (6-24 months)

Subaim 2.1: Evaluate pharmacological inhibition of IRE1 α or XBP1 as a therapeutic strategy in NSCLC. (3-18 months) (See Fig. 5-6)

Task: Determine the efficacy of single agent IRE1 α 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 HKP1 or 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 IRE1 α -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 B16 mice

Milestone(s) Achieved: 1) Treatment with IRE1 α selective small molecule inhibitors will boost anti-tumor immunity and impair tumor progression in NSCLC; 2) determine whether targeting IRE1 α 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 IRE1 α -XBP1 signaling modulates anti-tumor immunity.

Subaim 1.1 Identify IRE1 α /XBP1 regulated immunomodulators.

Task: Perform integrated pathway and gene set enrichment in order to identify known and novel candidate immunomodulators to be evaluated

To identify IRE1 α -XBP1-regulated genes, we performed RNA-seq analysis of IRE1 α ^{WT} and IRE1 α ^{KO} HKP1 cancer cells. We had previously reported on the identification of the IRE1 α -XBP1-PGE₂ axis in the HKP1 model, which we proposed to explore *in vivo*. Recently, we confirmed this in another Kras driven model (CMT167). *Ptges* mRNA transcript and secreted PGE₂ levels were reduced in *in vitro* Tg treated IRE1 α ^{KO} HKP1 cells compared with IRE1 α ^{WT} cells, confirming a direct link between IRE1 α and PGE₂ biosynthesis (**Fig. 1a-b**).

Furthermore, GSEA analysis of differentially regulated genes identified enrichment of type-I IFN-dependent gene signatures in IRE1 α ^{KO}, compared with their IRE1 α ^{WT} counterparts (**Fig. 2A-B**).

Furthermore, Ingenuity pathway analysis (IPA) of differentially regulated genes in IRE1 α ^{KO} vs IRE1 α ^{WT} tumor cells identified key upstream regulator s implicated in induction of type-I IFN signaling (**Fig. 2C**). This finding is relevant as inhibition of type-I IFN signaling in the TME has been associated with immunosuppression and immune escape in lung cancer (*Formenti et al., 2018; Wang et al., 2017*).

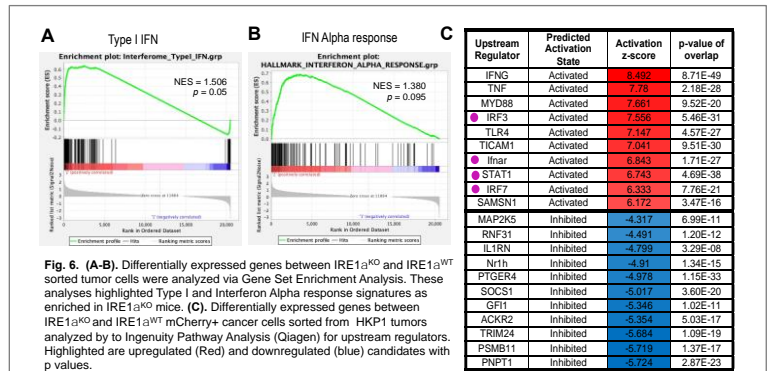
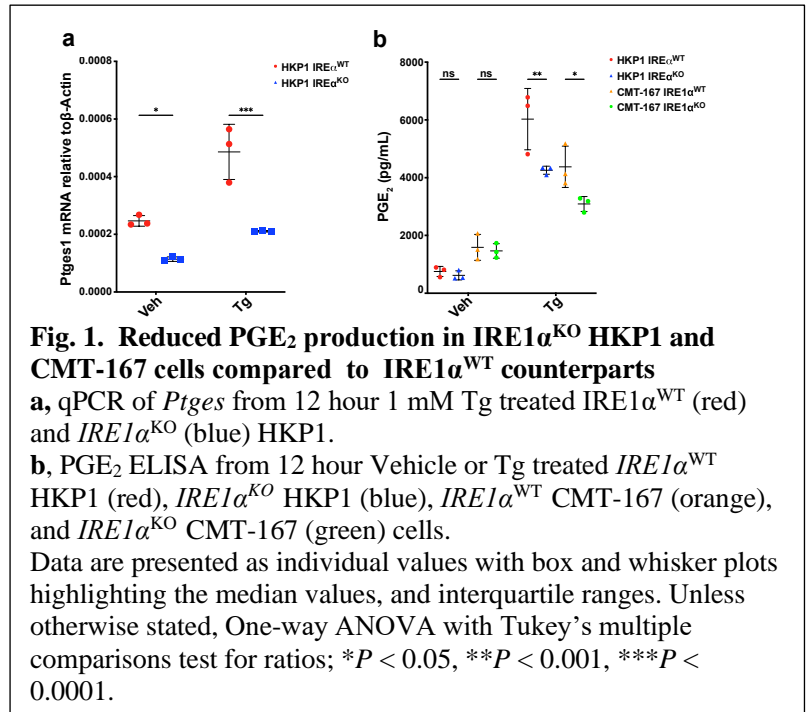


Fig. 2. (A-B). Differentially expressed genes between IRE1 α ^{KO} and IRE1 α ^{WT} sorted tumor cells were analyzed via Gene Set Enrichment Analysis. These analyses highlighted Type I and Interferon Alpha response signatures as enriched in IRE1 α ^{KO} mice. **(C)**. Differentially expressed genes between IRE1 α ^{KO} and IRE1 α ^{WT} mCherry+ cancer cells sorted from HKP1 tumors analyzed by Ingenuity Pathway Analysis (Qiagen) for upstream regulators. Highlighted are upregulated (Red) and downregulated (blue) candidates with p values.

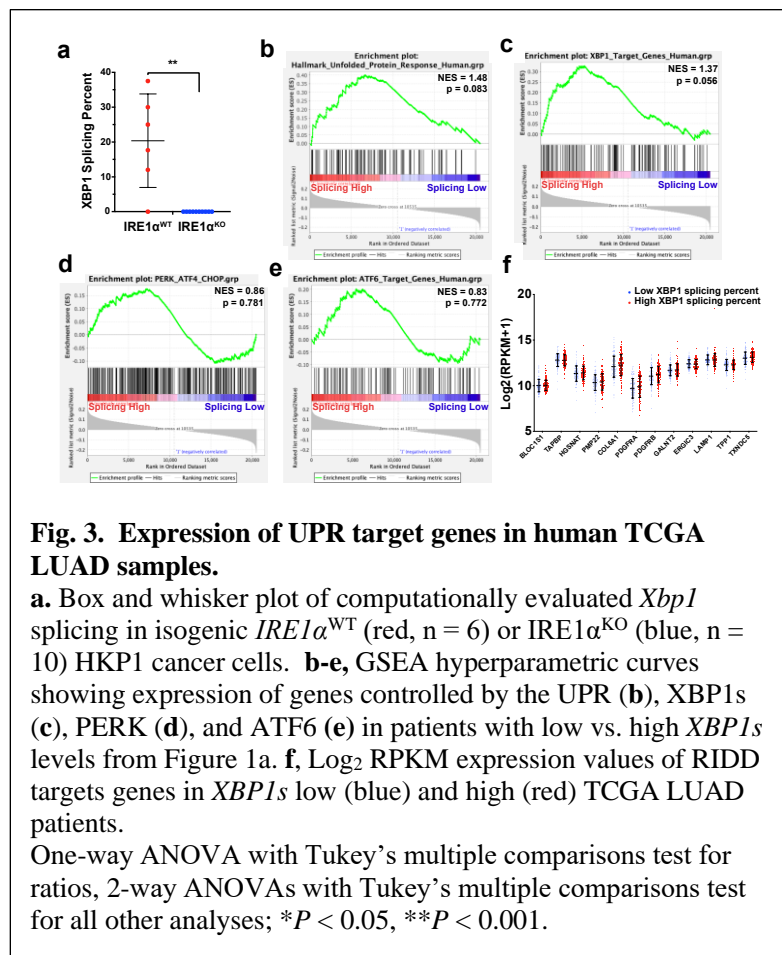
Importantly, type-I IFNs stimulate cDC1s to potently activate cytotoxic CD8⁺ T cells, thus enhancing adaptive immune control of cancer (Borden, 2019). We will test the hypothesis that IRE1 α -XBP1s signaling in the cancer cell restricts optimal expression and sensing of protective type-I IFNs in the TME.

We and others have demonstrated that IRE1 α signaling can govern mitochondrial homeostasis in various cell types via multiple mechanisms (Carreras-Sureda et al., 2019; Senft and Ronai, 2015; Song et al., 2018). Hence, we will determine if deletion of IRE1 α in the cancer cell promotes mitophagy, mitochondrial dysfunction, and/or release of mitochondrial DNA that could be directly sensed by the cancer cell or by neighboring myeloid cell populations in the TME, resulting in activation of innate sensors such as STING that drive production of type-I IFN. Accordingly, we will use STING-deficient cancer cells in the IRE1^{WT} or IRE1^{KO} backgrounds, as well as STING-deficient mice (JAX# 025805) implanted with either IRE1^{WT} or IRE1^{KO} HKP1 tumor cells, to discern the specific contribution of each compartment and assess the role of this sensor as a potential inducer of protective type-I IFN signaling in the TME of mice bearing IRE1 α -deficient HKP1 tumors.

Subaim 1.2: IRE1 α -XBP1 mediated gene signatures via RNA-seq in human NSCLC.

Since we were unable to embark on the in vivo studies, we decided to expand our analysis of the human TCGA LUAD analysis, which had shown that patients with high *XBP1s* were associated with significantly lower overall survival (OS) (Fig. 1 in the original proposal, $p < 0.0086$, *XBP1s* high median 37.6 months, *XBP1s* low median 58.3 months).

To determine the clinical relevance of IRE1 α -XBP1 signaling in NSCLC patients, we developed a new computational pipeline to specifically quantify the fraction of the spliced *XBP1* mRNA isoform (*XBP1s*) relative to the total *XBP1* transcript from RNA-seq data available in the TCGA database. Briefly, short reads were aligned to the *XBP1* unspliced transcript using Bowtie2 parameters that depenalize long gaps, followed by estimation of the fraction of spliced reads over read coverage around the *XBP1* splicing event. We demonstrated the sensitivity of this strategy using RNA-seq data from isogenic IRE1 α ^{WT} and IRE1 α ^{KO} cell lines (Fig. 3a). To demonstrate that the difference in survival was specific to IRE1 α -XBP1 axis but not due to a broader UPR response, we performed Gene Set Enrichment Analysis (GSEA), comparing the top and bottom *XBP1s* tertiles against UPR signatures derived from the Molecular Signatures Database (MSigDB), and Harmonizome. Both the Hallmark UPR signature and *XBP1s* target genes were enriched in the *XBP1s* high patient cohort (Fig. 3b-c), whereas genes controlled by other sensors of ER stress including protein kinase RNA (PKR)-like ER Kinase (PERK), activating transcription factor 6 (ATF6) (Cubillos-Ruiz et al., 2017)



were not significantly enriched (Fig. 3d-e). Additionally, we did not observe differential expression of Regulated IRE1 α -Dependent Decay (RIDD) targets between these groups (Fig. 3f). These findings demonstrate *XBPIs* levels as prognostic of reduced survival in human NSCLC.

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 3 (NCE), we will perform the following (as we have obtained IACUC approval, pending ACURO approval, and COVID restrictions have been majorly lifted in NYC):

- 1) Ectopic expression of Ptges and XBP1 in IRE1a KO HKP1 tumors to rescue phenotypes in vivo.
- 2) Investigate the consequences of modulated PGE₂ levels on the immune landscape of IRE1α^{KO} HKP1 tumors.
- 3) Use IRE1a inhibitor to determine impact on HKP1 tumor progression
- 4) Explore IRE1α-XBP1s regulation of type-I IFN responses.

4. IMPACT: Describe 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:*

None.

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.

Our goal in year 2 was to perform in vivo mouse experiments to expand upon the in vitro findings in year 1. However, due to COVID related delays in mouse breeding and delays in animal approval, we expect to accomplish these experiments during the NCE (year 3).

- 1) Ectopic expression of Ptges and XBP1 in IRE1a KO HKP1 tumors to rescue phenotypes in vivo.
- 2) Investigate the consequences of modulated PGE₂ levels on the immune landscape of IRE1α^{KO} HKP1 tumors.
- 3) Use IRE1a inhibitor to determine impact on HKP1 tumor progression.

The finding that IRE1αKO also regulated type I IFN will also allow us to explore this in vivo

- 4) Explore IRE1α-XBP1s regulation of type-I IFN responses.

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.

N/A

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).*

A manuscript is in preparation.

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 research grant R01 CA257254-01A1 from the NIH NCI entitled, “Copper mediated metabolic reprogramming and ECM alterations in TNBC metastasis.” Dates are from 05/01/2021 – 04/30/2026. He is spending 15% effort (1.8 calendar months).

Dr. Mittal received a research grant I14-0036 from the Starr Cancer Consortium entitled, “Examining the link between EZH2 and chromosomal instability in metastatic breast cancer.” Dates are from 01/01/2021 – 12/31/2022. He is spending 10% effort (1.2 calendar months).

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); and*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

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

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

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

Borden, E.C. (2019). Interferons α and β in cancer: therapeutic opportunities from new insights. *Nat Rev Drug Discov* 18, 219-234. 10.1038/s41573-018-0011-2.

Carreras-Sureda, A., Jaña, F., Urra, H., Durand, S., Mortenson, D.E., Sagredo, A., Bustos, G., Hazari, Y., Ramos-Fernández, E., Sassano, M.L., et al. (2019). Publisher Correction: Non-canonical function of IRE1 α determines mitochondria-associated endoplasmic reticulum composition to control calcium transfer and bioenergetics. *Nat Cell Biol* 21, 913. 10.1038/s41556-019-0355-9.

Cubillos-Ruiz, J.R., Bettigole, S.E., and Glimcher, L.H. (2017). Tumorigenic and Immunosuppressive Effects of Endoplasmic Reticulum Stress in Cancer. *Cell* 168, 692-706. 10.1016/j.cell.2016.12.004.

Formenti, S.C., Rudqvist, N.P., Golden, E., Cooper, B., Wennerberg, E., Lhuillier, C., Vanpouille-Box, C., Friedman, K., Ferrari de Andrade, L., Wucherpennig, K.W., et al. (2018). Radiotherapy induces responses of lung cancer to CTLA-4 blockade. *Nat Med* 24, 1845-1851. 10.1038/s41591-018-0232-2.

Senft, D., and Ronai, Z.A. (2015). UPR, autophagy, and mitochondria crosstalk underlies the ER stress response. *Trends Biochem Sci* 40, 141-148. 10.1016/j.tibs.2015.01.002.

Song, M., Sandoval, T.A., Chae, C.S., Chopra, S., Tan, C., Rutkowski, M.R., Raundhal, M., Chaurio, R.A., Payne, K.K., Konrad, C., et al. (2018). IRE1 α -XBP1 controls T cell function in ovarian cancer by regulating mitochondrial activity. *Nature* 562, 423-428. 10.1038/s41586-018-0597-x.

Wang, X., Schoenhals, J.E., Li, A., Valdecanas, D.R., Ye, H., Zang, F., Tang, C., Tang, M., Liu, C.G., Liu, X., et al. (2017). Suppression of Type I IFN Signaling in Tumors Mediates Resistance to Anti-PD-1 Treatment That Can Be Overcome by Radiotherapy. *Cancer Res* 77, 839-850. 10.1158/0008-5472.CAN-15-3142.