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TITLE: Treatment and Response Targets for Helicobacter-Associated Gastric Cancer

PRINCIPAL INVESTIGATOR: Dr. Marygorret Obonyo, PhD

CONTRACTING ORGANIZATION: University of California, San Diego

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6. AUTHOR(S)

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

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14. ABSTRACT

The project deals with identifying treatment and response targets for *Helicobacter*-associated gastric cancer. The purpose is to discover novel druggable targets by testing efficacy of approved compounds. An additional purpose is to identify immune related genes as predictors of treatment response. To accomplish the goals of our project we are using fresh gastric tumor tissue samples harvested from patients undergoing gastrectomy to generate patient-derived xenograft (PDX) and patient-derived orthotopic xenograft (PDOX) models. In addition, the work makes use of gastric cancer patient samples stored at our UCSD's Moores Cancer Center Biorepository core. We have successfully established a gastric cancer PDX model and show that tumor gastric tissues in the PDX model express the same genes as in the original tumor. We have started drug targeting studies by testing efficacy of approved compounds as promising candidates for gastric cancer treatment. Figure 1 (below) shows targeting of a protein from a differentially expressed gene (DEG) that exhibited overexpression in our accelerated model (**Fig. 2A**) and was similarly elevated in human gastric cancer biopsies (**Fig. 2B**) - proteasome subunit beta type 8 (PSMB8).

15. SUBJECT TERMS					
Helicobacter pylori, gastric cancer, patient-derived xenograft (PDX), patient-derived orthotopic xenograft (PDOX), differentially expressed genes (DEGs).					
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1. INTRODUCTION:

The project deals with the identification of treatment and response targets of *Helicobacter*-associated gastric cancer. This work leverages our findings from our mouse model of gastric cancer(1, 2) to discover novel druggable targets by testing efficacy of approved compounds and to identify predictors of treatment response using freshly obtained patient gastric tissue samples, patient-derived xenograft (PDX), and patient-derived orthotopic xenograft (PDOX) models. In addition, the work makes use of gastric cancer patient samples stored at the tissue biobank at the UCSD's Moores Cancer Center Biorepository core.

2. KEYWORDS:

Helicobacter pylori, gastric cancer, patient-derived xenograft (PDX), patient-derived orthotopic xenograft (PDOX), differentially expressed genes (DEGs), proteasome subunit beta type 8 (PSMB8).

3. ACCOMPLISHMENTS:

What were the major goals of the project?

3.1: The major goals of the project are to deliver drugs to slow or stop the progression of gastric cancer and to determine patients likely to respond well to a specified drug.

What was accomplished under these goals?

3.2:

Targeted therapy experiment in gastric epithelial-derived tumors in a xenograft model.

We have initiated targeted therapy experiments using tumor-derived gastric cancer cell lines within a xenograft model. Specifically, we targeted one of the DEGs, PSMB8 that was elevated in both human gastric biopsies and our accelerated model of gastric cancer compared to the standard model. Human gastric epithelial cell-derived tumors were implanted subcutaneously in nude mice and treated with 5-fluorouracil (5-FU) (50 mg/kg), carfilzomib (5mg/kg), or the combination of the two drugs by intraperitoneal injections (IP). Control nude mice receive phosphate buffered saline (PBS) in 2% dimethylsulfoxide (DMSO) by IP. Tumors were measured twice a week. **Fig. 1** illustrates the progression of tumors in untreated and treated conditions. Specifically, we targeted a protein from the DEGs that exhibited overexpression in our accelerated model (**Fig. 2A**) and was similarly elevated in human gastric cancer biopsies (**Fig. 2B**) - proteasome subunit beta type 8 (PSMB8). By exploring available pharmaceutical databases, we identified carfilzomib as a potential drug capable of targeting this protein. The current results strongly suggest that PSMB8 plays a role in driving disease progression, as evidenced by the retardation of tumor growth upon treatment with carfilzomib. While these results are preliminary, they are highly promising. Importantly, our targeted treatment has demonstrated greater efficacy in inhibiting tumor growth compared to the standard of care drug, 5-FU. Our next steps will involve further validation of the target, followed by carfilzomib treatment studies conducted in patient-derived xenograft and orthotopic models. Importantly, we have observed elevated levels of PSMB8 in patient gastric biopsies.

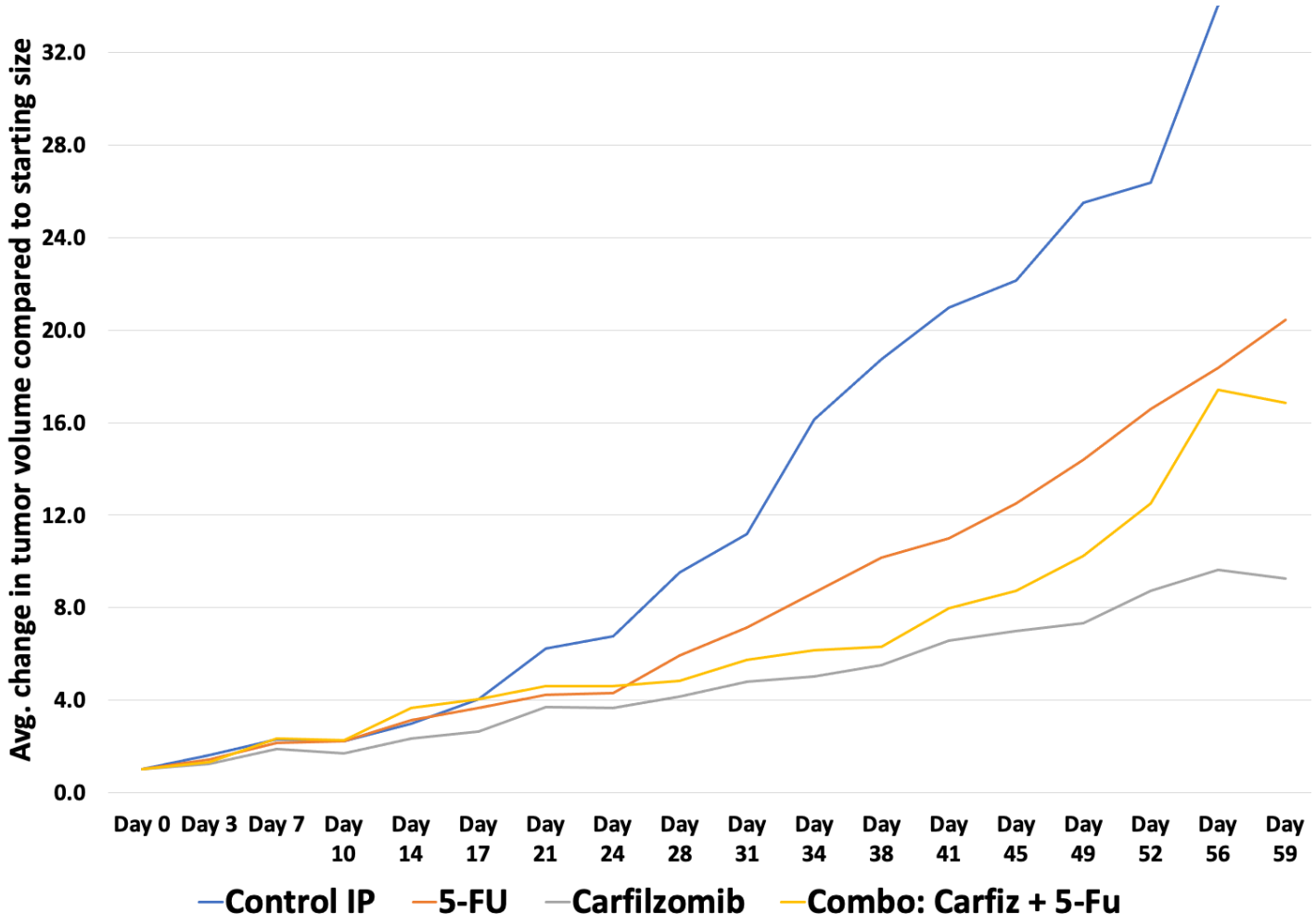

Figure 1. Targeting of one of the DEGs, PSMB8 in a xenograft model. Human gastric epithelial cell-derived tumors were implanted subcutaneously in nude mice and treated with 5-FU (50 mg/kg), carfilzomib (5mg/kg), or the combination of the two drugs by intraperitoneal injections (IP). Control nude mice receive PBS in 2% DMSO by IP. 8 nude mice were randomly assigned to treatment group.

Overexpression of DEGs in our accelerated gastric cancer model (A) mirrors DEGs expression in gastric tissue samples from *H. pylori*-induced chronic atrophic gastritis (B).

DEGs that were overexpressed in our accelerated *Helicobacter* infection model (**Fig. 2A**) were indeed similarly elevated in gastric tissue samples from *H. pylori*-induced chronic atrophic gastritis patients (**Fig. 2B**). This finding of similarly highly expressed DEGs between our accelerated model and human gastric tissue samples from patients infected with *H. pylori* contributes to the validation of our mouse model to simulate *H. pylori* infection disease progression in human. For the targeted therapy study above, we selected PSMB8 that was elevated in both our accelerated model and gastric tissue samples from patients with *H. pylori-induced* chronic atrophic gastritis. For both the mouse models of gastric cancer (standard and accelerated), expression of DEGs was identified by RNA sequencing (RNA-seq) analysis of mouse stomach tissue sections following infection with *H. felis* for 3 months. For H. pylori-induced chronic atrophic gastritis heatmap, we reanalyzed published microarray data set of gastric tissue from uninfected and *H. pylori*-infected patient with atrophic gastritis (GSE27411 data sets). The data showed overexpression of DEGs in the stomach of patients with *H. pylori*-induced chronic atrophic gastritis.

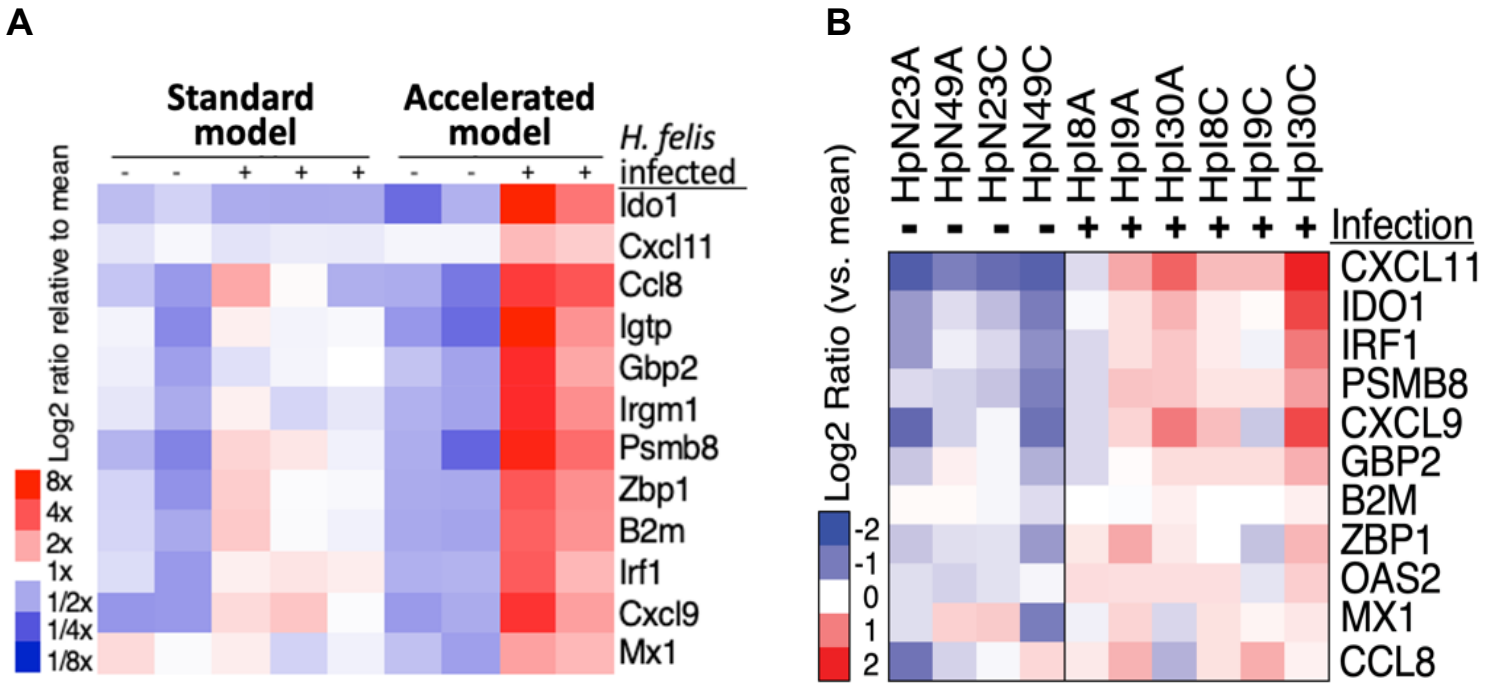

Figure 1. (A) The accelerated model exhibits high expression of DEGs, which was associated with poor disease outcome. The heat map is a representation of select DEGs to show differential expression levels between the models. For both models, expression of DEGs was identified by RNA-seq analysis of mouse stomach tissue sections following infection with *H. felis* for 3 months. Each column represents a mouse either infected with *H. felis*, denoted as "+" or left uninfected, denoted by "-". **(B)** Reanalysis of published microarray data set of gastric tissue from uninfected and *H. pylori*-infected patient with atrophic gastritis showed overexpression of DEGs in the stomach of patients with *H. pylori*-induced chronic atrophic gastritis. GSE27411 data sets. Samples from *H. pylori*-infected patients are denoted by "+" (n=6) and uninfected by "-" (n=4).

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

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report.

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

We are currently optimizing drug targeted treatment conditions using human gastric epithelial cell-derived tumors with the next step to use human gastric biopsy samples. In addition, to the current protein, PSMB8, we will also target proteins of DEGS that were elevated in both our accelerated model and patient gastric biopsies. These targeted therapy experiments will be followed by further validation of the target. These experiments will be conducted in patient-derived xenograft and orthotopic models, which were generated in the first years of the project.

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?

The current results strongly suggest that PSMB8 plays a role in driving disease progression, as evidenced by the retardation of tumor growth upon treatment with carfilzomib. Importantly, our targeted treatment has demonstrated greater efficacy in inhibiting tumor growth compared to the standard of care drug, 5-FU.

What was the impact on other disciplines?

Our results showing carfilzomib inhibits gastric epithelial cell-derived tumor growth could be used to inhibit tumor growth in other cancers.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

There are no changes in the approach and therefore, nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Due to the COVID-19 pandemic we were not able to harvest many gastric tumor biopsy samples as we had planned. More patients have started scheduling gastric surgeries and therefore we anticipate we will collect more patient gastric cancer biopsy samples. We therefore requested for a no-cost-extension to have more time to collect these gastric biopsy samples. We are grateful that the no-cost-extension was granted.

Changes that had a significant impact on expenditures

Nothing to Report.

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

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to Report

Books or other non-periodical, one-time publications.

Nothing to Report.

Other publications, conference papers and presentations.

- The San Diego Digestive Diseases Research Center (SDDRC): Annual Symposium. February 11th, 2022.
- UCSD, Division of Pediatric Host-Microbe Systems & Therapeutic: January 9, 2023.
- The University of Arizona Cancer Center, Collaborative Cancer Grand Rounds Presentation: January 20, 2023.
- UCSD Moores Cancer Center: 2023 Immune Oncology Symposium. June 9th, 2023.

- **Website(s) or other Internet site(s)**

Nothing to Report.

- **Technologies or techniques**

Nothing to Report.

- **Inventions, patent applications, and/or licenses**

Nothing to Report.

- **Other Products**

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
What individuals have worked on the project?

Name:	Marygorret Obonyo
Project Role:	PI
Nearest person month worked:	3.6
Contribution to Project	Dr. Obonyo was involved in the design of experiments and overall supervision of the project.
Funding Support	
Name:	Michael Bouvet
Project Role:	Investigator
Nearest person month worked:	0.6
Contribution to Project	Dr. Bouvet alongside his lab staff assistant, Siamak Amirfakhri have generated the gastric cancer patient-derived orthotopic xenograft (PDOX) model.
Funding Support	
Name:	Kaitlyn Kelly
Project Role:	Investigator
Nearest person month worked:	0.6
Contribution to Project	Dr. Kelly has provided both involved and uninvolved patient gastric cancer specimens.
Funding Support	
Name:	Siamak Amirfakhri
Project Role:	Staff Research Assistant
Nearest person month worked:	2.4
Contribution to Project	Under supervision of Dr. Bouvet, Siamak Amirfakhri has generated the gastric cancer PDOX model.
Funding Support	
Name:	Michael Turner
Project Role:	MD Fellow
Nearest person month worked:	1.2
Contribution to Project	Dr. Turner has performed work related to the generation and maintenance of the gastric cancer PDOX model.
Funding Support	T32 NIH Training grant
Name:	Jonathan Hernandez
Project Role:	Staff Research Assistant
Nearest person month worked:	6

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

Nothing to Report.

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: N/A

References:

1. Banerjee A, Thamphiwatana S, Carmona EM, Rickman B, Doran KS, Obonyo M. 2014. Deficiency of the myeloid differentiation primary response molecule MyD88 leads to an early and rapid development of Helicobacter-induced gastric malignancy. Infect Immun 82:356-63.
2. Lozano-Pope I, Sharma A, Matthias M, Doran KS, Obonyo M. 2017. Effect of myeloid differentiation primary response gene 88 on expression profiles of genes during the development and progression of Helicobacter-induced gastric cancer. BMC Cancer 17:133.