AWARD NUMBER: **W81XWH-17-1-0450**

TITLE: **Rational Therapies for diffuse-type gastric cancer**

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REPORT DATE: **DECEMBER 2019**

TYPE OF REPORT: **FINAL**

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
DECEMBER 2019	FINAL	08/15/2017-08/14/2019

4. TITLE AND SUBTITLE
Rational Therapies for diffuse-type gastric cancer

5a. CONTRACT NUMBER
W81XWH-17-1-0450

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
Year 2 progress report in lieu of final report due to NCE request.

14. ABSTRACT:
Diffuse-type gastric cancer (DGC) is a devastating disease that is associated clinically with linitis plastic, which is characterized by diffuse infiltration of the gastric wall. Histologically, the DGC consists of cells displaying loss of hemophilic cell-cell interactions, signet-ring features and diffuse cell scattering into normal tissues. Symptoms occur late, frequently in form of early satiety resulting from cancer-related stiffness of the gastric wall. Clinical management of the disease is particularly challenging because it occurs significantly more frequently in younger patients compared to the intestinal type, does not respond well to chemotherapy and no effective targeted therapies are known. As a result of these factors, the prognosis for patients with the disease is poor: the median survival for patients with DGC is 17 months after surgical resection with curative intent compared to 129 months for patients with intestinal type. Similarly, DGC has been found to be associated with poor response to neo-adjuvant chemotherapy. Thus, there is an urgent need for novel treatment approaches for this devastating disease.

15. SUBJECT TERMS
Gastric cancer, Diffuse subtype

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	6	19b. TELEPHONE NUMBER *(include area code)*

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1. Introduction

Diffuse-type gastric cancer (DGC) is a devastating disease that is associated clinically with linitis plastic, which is characterized by diffuse infiltration of the gastric wall. Histologically, the DGC consists of cells displaying loss of hemophilic cell-cell interactions, signet-ring features and diffuse cell scattering into normal tissues. Symptoms occur late, frequently in form of early satiety resulting from cancer-related stiffness of the gastric wall. Clinical management of the disease is particularly challenging because it occurs significantly more frequently in younger patients compared to the intestinal type, does not respond well to chemotherapy and no effective targeted therapies are known. As a result of these factors, the prognosis for patients with the disease is poor: the median survival for patients with DGC is 17 months after surgical resection with curative intent compared to 129 months for patients with intestinal type. Similarly, DGC has been found to be associated with poor response to neo-adjuvant chemotherapy. Thus, there is an urgent need for novel treatment approaches for this devastating disease.

Molecularly, diffuse-type gastric cancer is characterized by loss of expression of the adherens-junction molecule E-cadherin. Germline mutations in the E-cadherin gene result in hereditary diffuse gastric cancer syndrome with a life-time risk of gastric cancer of 40-80% [9]. DGC also occurs in patients chronically infected with H. pylori and somatic mutations of CDH1 are frequently found [10]. Next-generation DNA sequencing analyses and RNA expression profiling of large series of gastric cancer confirm the crucial role of E-cadherin in DGC, which is consistently non-functional in this disease type either as a consequence of mutations or promoter hypermethylation [6]. Point mutations frequently lead to disruption of calcium-binding or inhibition of dimerization. Both processes are crucial for the molecule's adhesive functions x[11]. Truncating mutations or deep chromosomal loss result in loss of E-cadherin protein expression, as does promoter hypermethylation. The latter mechanisms also is frequently the providing the "second hit" in cancers heterozygous for an inactivatingCDH1 mutations [12]. Tumors demonstrating loss of E-cadherin expression are characterized by an overall low mutational burden and low abundance of DNA copy number changes and therefore have been classified as genomically stable (GS subtype) by the The Cancer Genome Atlas Research Network (TCGA; [4]). Gastric cancers with loss of E-cadherin demonstrate evidence for epithelial-to-mesenchymal transition, a phenotypic reprogramming of cells characterized by over-expression of transcription factors such as Snail, Slug, Twist, ZEB1 and ZEB2 and over-expression of vimentin [6]. In agreement with these molecular changes, DGC cells show increased mobility and invasiveness [13-15]. We

The purpose of this research is 1) to use CRISPR-based strategies to generate isogenic gastric cell lines with wildtype or mutant CDH1 which can be used to explore the signaling networks that regulate cytoskeletal changes in CDH1 mutant cells 2) to assess the cellular effects of pharmacological inhibitors on key pathways involved in diffuse-gastric cancer; and 3) to test

the anti-tumor efficacy of these inhibitors in PDX models. Through these experiments we hope to provide sufficient and convincing preclinical data to stimulate expeditious design of clinical trials.

2. Keywords.

Gastric cancer, Diffuse subtype

3. Accomplishments

- **What were the major goals of the project?**

  1. Generate CDH1 gene-edited cell lines

  2. Evaluate the effect of pharmacological inhibitors in diffuse gastric cell line models
  3. In vivo efficacy studies in pre-clinical PDX models

- **What was accomplished under these goals?**

Goal 1.

Previously we accomplished in generating a panel of 7 gastric cell line models: MKN7, AGS, KATO-III, NCI-SNU-1, NCI-SNU-5, NCI- SNU16 and NCI-N87 to which we have determined the copy number and mutational status of CDH1 and identified a single base pair insertion in AGS cells prematurely terminates CDH1 in exon 12.

Recent months we screened MEK and PARP inhibitors to examine EC50 and now need to determine alterations in cellular signaling in CDH1 edited cells

Goal 2.

This year we started to perform efficacy and mechanistic studies of the effect of MEK, TGF-beta and additional inhibitors in diffuse-type gastric cancer cell lines. This work is underway, but not yet completed due to personnel issues attendant to leadership changes in the grant last year.

Goal 3:

We recently have identified several potential CDH1-mutant PDX models based on exome sequencing and microarray expression analysis in collaboration with Charles River Laboratories. We reviewed mutation data, RNA expression data, and IHC data. We determined GXF 602 was a PDX model with a frame shift mutation in CDH1, no WT allele and the GXIF and GXA lines were selected based on RNA expression data wherein the CDH1 transcript was low.

We are testing therapeutics on confirmed CDH1-mutant PDX models in the coming 12 months as proposed in this aim with the following PDX experiment:

- 2 models (GXF602, GXA SMTCA18)
- dosed every other day for 7 weeks with vehicle or 25mg/kg foretinib
- 8 animals per arm.

This work is ongoing at Charles River.

We have also identified PDX #'s DQ and AR from MSKCC as harboring deleterious CHD1 mutations. Additionally PDX#'s DS and DV harbor deep deletions in CDH1. In the next month we plan to test these lines with foretinib as well, and compare them to wt controls at MSKCC.

4. IMPACT

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

N/A

- **How were the results disseminated to communities of interest?**

N/A

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

Test isogenic cell line models for differences in sensitivity to targeted agents (MEK, TGFb, ROS1)
Perform CDH1-mutant PDX models for xenograft studies with foretinib.

- **What was the impact on technology transfer?**

Noting 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**

None

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

The project experienced some delay secondary to leadership changes in the first year of the grant. Dr. Korn, the original PI, left the institution for a position in industry (Caris Biosceinces). Dr. Collisson recruited new personnel to work on the project and it is going well. The hiring environment has been a challenge in San Francisco and delays were mostly due to leadership changes and delay in hiring.

- **Changes that had a significant impact on expenditures**

We have had trouble hiring in the bay Area for this project due to the competitive labor economy for biotech workers. Recently a new Scientist, Henry (Hua) Chen has been recruited to work on this project.

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

Nothing to report

6. PRODUCTS

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report