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

TITLE: **Rational Therapies for Diffuse-Type Gastric Cancer**

PRINCIPAL INVESTIGATOR: **Eric Collisson, MD**

CONTRACTING ORGANIZATION: **The Regents of the University of California**
San Francisco, CA 94103

REPORT DATE: **Sept 2018**

TYPE OF REPORT: **ANNUAL**

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
Sept 2018	ANNUAL	15 Aug 2017 - 14 Aug 2018

4. TITLE AND SUBTITLE

Rational Therapies for Diffuse-Type Gastric Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-1-0450

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Dr. Eric A. Collisson, MD

E-Mail:

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
1855 FOLSOM ST STE 425
SAN FRANCISCO CA 94103-4249

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

14. ABSTRACT

We have collected a panel of 7 gastric cell line models: MKN7, AGS, KATO-III, NCI-SNU-1, NCI-SNU-5, NCI-SNU16 and NCI-N87. Using data available through CCLE and Cosmic Databases {Garnett, 2012 #76} {Barretina, 2012 #1}, we 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. We have designed and constructed CRISPR reagents to correct this mutation and restore CDH1 function. This isogenic cell line pair will be used to investigate the signaling networks in CDH1-deficient cells compared to CDH1-proficient lines.

15. SUBJECT TERMS

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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"Rational therapies for diffuse-type gastric cancer" from DOD US Army Med. Res. Acq. Activity
Progress report:

- Award No: 1710450
- Grant#W81XWH-17-1-0450
- Principal Investigator: Collisson, Eric A.

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 promoterhypermethylation [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. Thelatter 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 lowmutational 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.

We have collected a panel of 7 gastric cell line models: MKN7, AGS, KATO-III, NCI-SNU-1, NCI-SNU-5, NCI-SNU16 and NCI-N87. Using data available through CCLE and Cosmic Databases {Garnett, 2012 #76} {Barretina, 2012 #1}, we 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. We have designed and constructed CRISPR reagents to correct this mutation and restore CDH1 function. This isogenic cell line pair will be used to investigate the signaling networks in CDH1-deficient cells compared to CDH1-proficient lines.

Cell Lines	Tissue of Origin	E-cadherin status	mRNA expression	Mutation status
AGS	stomach	Null	5.761766	c.1732_1733insC (Frame_Shift_Ins)
SNU1	stomach	Null	4.547656	
SNU5	stomach	Null	4.92593	c.687_splice (Splice_Site_SNP)
KATOIII	stomach	WT	9.104514	c.461G>C (Missense_Mutation)
MKN7	stomach	WT	11.22368	
N87	stomach	WT	9.494066	
SNU16	stomach	WT	10.17294	

Goal 2.

No progress.

Goal 3:

In conversations with Charles River Laboratories and Memorial Sloan Kettering, we have identified several potential CDH1-mutant PDX models based on exome sequencing and microarray expression analysis. We are currently working to confirm CDH1 loss by IHC in these models.

IMPACT

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

How were the results disseminated to communities of interest?

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

Engineer isogenic cell line models
Confirm CDH1-mutant PDX models for xenograft studies.

What was the impact on technology transfer?

Noting to report

What was the impact on society beyond science and technology?

Nothing to report.

CHANGES/PROBLEMS
Changes in approach and reasons for change

None

Actual or anticipated problems or delays and actions or plans to resolve them
Due to the change in PI, we have had some unexpected delays in finding full time scholars to work on this project. We have identified two candidates and have offered employment to one.

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

Nothing to report

PRODUCTS

• Publications, conference papers, and presentations

Books or other non-periodical, one-time publications.
Nothing to report

Other publications, conference papers, and presentations
Nothing to report

• Website(s) or other Internet site(s)
Nothing to report

• Technologies or techniques

• Inventions, patent applications, and/or licenses
Nothing to report

• Other Products
Nothing to report