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PRINCIPAL INVESTIGATOR: Eric Collisson, MD

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

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

E-Mail: eric.collisson@ucsf.edu

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THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
1855 FOLSOM ST STE 425
SAN FRANCISCO CA 94103-4249

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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

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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].

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?**

  Specific Aim 1: To generate and characterize genetically defined cell line models of diffuse type gastric cancer

  Major Task 1: Generate CDH1 gene-edited cell lines - COMPLETED
  - · Generation of CDH1-edited cell lines
  - · Determine alterations in cellular signaling in CDH1 edited cells

  Specific Aim 2: To evaluate efficacy of inhibitors of TGF-beta, MEK and additional targets in cell line models of diffuse type gastric cancer

  Major Task 2: Evaluation of drug activity in diffuse-type gastric cancer cell lines

  Specific Aim 3: Test therapeutics identified in Aim 2 in patient derived xenograft models

  Major Task 3: In vivo efficacy and pharmacodynamics of inhibitors of MEK, TGF-beta and additional inhibitors in patient- derived xenograft models.

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

Goal 1.

Completed.

Goal 2.

No progress. We were not able to re-introduce CDH1 into CRSPR-edited gastric cancer cell lines. We hypothesize that this may be due to the tumor suppressor function of the CDH1 protein.

Goal 3:

Experiments in existing patient-derived xenograft models of diffuse-type gastric cancer. Assessment of pharmacodynamics markers of drug activity in samples from xenograft models.

We have generated a collection of 113 patient-derived xenograft (PDX) models from patients with gastric, esophageal and gastroesophageal junction cancers. Of these 113 PDXs, 67 PDXs were derived from 57 patients with HER2-positive tumors, including patients with metastatic (43 patients, 75%) and early stage/locally advanced (14 patients, 25%) cancers at the time of PDX collection. The PDXs were generated from either surgical resections or biopsies (Figure 1A) using tissue from the primary esophagogastric tumor or a metastatic site (Figure 1B). In addition, for some HER2-positive patients, we have derived two (9 patients) or three (1 patient) PDXs from the same patient at different timepoints within the treatment course. The large majority of PDXs from patients with metastatic HER2-positive cancers were generated from patients with treatment-refractory disease with progression on trastuzumab and chemotherapy containing regimens (Figure 1C). Other PDXs were derived from patients with newly diagnosed metastatic disease or with ongoing responses to trastuzumab-containing therapy. Moreover, we have generated several HER2-positive esophagogastric cancer PDXs from patients with localized disease, including PDXs from untreated tumors and tumors exposed to preoperative chemotherapy or chemoradiation (Figure 1D).

We have performed targeted sequencing of most of the PDXs using the MSK-IMPACT next-generation sequencing platform, with the latest version including 468 genes. The majority of these PDXs have corresponding clinical sequencing from patient tumors, allowing for analysis of genomic fidelity between patient and PDX samples. Frequently co-occurring alterations with HER2 amplifications include *TP53* mutations, cell-cycle gene alterations (i.e. *CDK12*, *CCDN3*, *CDKN2B*, *CCNE1*) and receptor tyrosine kinase alterations (such as *EGFR*), consistent with patient sequencing data.

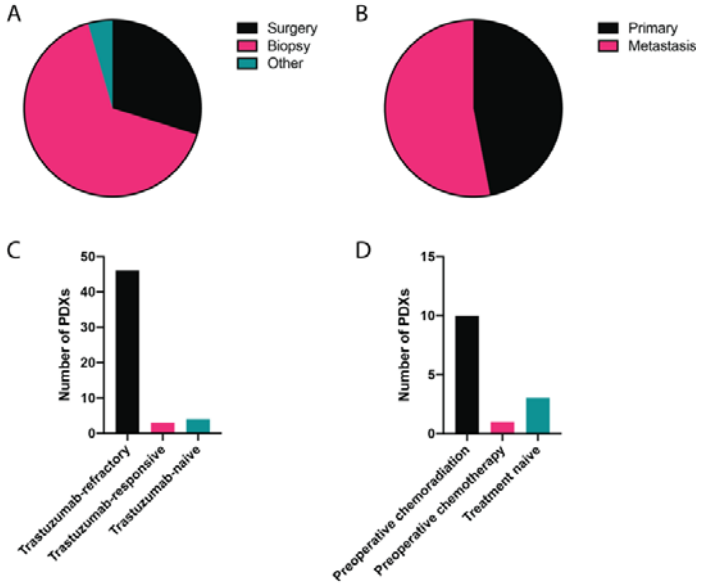

Figure 1. Overview of HER2-positive esophagogastric cancer PDXs. (A) Procedure for generation of PDXs. (B) Tissue origin (primary tumor or metastasis) for PDXs. (C) Treatment history with trastuzumab for HER2-positive PDXs derived from patients with metastatic disease. (D) Preoperative treatment history for PDXs derived from patients with localized HER2-positive tumors.

Table 1. Patient and PDX characteristics

Characteristic	N (%)
Gender (%)	
Women	11 (19)
Men	46 (81)
Anatomic tumor location (%)	
Gastroesophageal junction	18 (31)
Esophagus	18 (31)
Stomach	22 (38)
Stage at initial PDX collection (%)	
IV	43 (75)
III	8 (14)
II	5 (9)
I	1 (2)
Number of PDX per patient (%)	
1	46 (81)
2	9 (16)
3	1 (2)
Sample Type (%)	
Primary	31 (47)
Metastasis	33 (53)
Procedure Type	
Surgery	20 (30)
Biopsy	44 (66)
Other	3 (4)

4. IMPACT

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

N/A

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

We have discussed these studies at the Helen Diller cancer Center and plan submitting studies for publication in the near future.

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

We plan a draft publication by March 2021.

- **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 only minimal progress in **Goal 2** due to personnel. The hiring environment has been a challenge due to Covid19. Further, we had difficulty establishing cell lines with re-introduced CDH1 for likely biological reasons.

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

N/A

- **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