AWARD NUMBER: W81XWH-21-1-0623

TITLE: Identifying Drivers of Therapy Resistance Within the Tumor Microenvironment of Esophageal Adenocarcinoma

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REPORT DATE: October 2023

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2023	Annual	01Sep2022-31Aug2023

4. TITLE AND SUBTITLE

Identifying Drivers of Therapy Resistance Within the Tumor Microenvironment of Esophageal Adenocarcinoma

5a. CONTRACT NUMBER
W81XWH-21-1-0623

5b. GRANT NUMBER
CA200572

5c. PROGRAM ELEMENT NUMBER
n/a

5d. PROJECT NUMBER
n/a

5e. TASK NUMBER
n/a

5f. WORK UNIT NUMBER
n/a

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8. PERFORMING ORGANIZATION REPORT NUMBER
n/a

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development 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

Gastroesophageal adenocarcinoma is a fast-rising malignancy with a generally poor outcome and a lack of targeted therapy options. Here, we aim to uncover the mechanisms through which the tumor microenvironment (TME) mediates resistance to chemotherapy, with goals of identify novel biomarkers for patient stratification as well as elements within the TME that can be targeted to enhance the efficacy of existing and newly emerging tumor-directed therapies. This will be accomplished via both retrospective and prospective analyses, integrating transcriptomic, genomic, and proteomic analyses with patient-relevant model systems (patient-derived organoids), innovative coculture platforms and high-throughput drug screens.

15. SUBJECT TERMS
Esophageal cancer; chemoresistance; tumor microenvironment; patient-derived organoids; single-cell RNA sequencing; moel systems; biomarkers

16. SECURITY CLASSIFICATION OF: n/a			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	9	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **Introduction.** Gastro-esophageal cancer (GEA) is a disease with increasing incidence and generally poor outcome. Available standard-of-care therapy consists of docetaxel-based chemotherapy (with or without radiation) in the presurgical (neoadjuvant) and post-surgical (adjuvant) settings. However, ~40% of cases are resistant to this therapy up front, and ca. half of those who do respond develop resistance later. Given the paucity of targets within the tumor (driven by high heterogeneity and non-genetic alterations), we propose that targeting tumor-microenvironment interactions driving chemoresistance can be an effective approach. To address this goal, this project will 1. Conduct retrospective analyses of tumor microenvironment (TME) in cases with known response to therapy; 2. Prospectively collect and deeply characterize samples obtained across the neoadjuvant treatment continuum (pre-, mid- and post-treatment), while simultaneously creating avatars (patient-derived organoids; PDOs) as model systems for each sample; 3. Validate TME-inclusive drug screening strategies to identify salvage therapies for chemo-resistant cases. Our overall goal is to investigate and position TME-direct complementary therapies as an effective modality to defeat resistance to therapy in GEA.
2. **Keywords.** Esophageal cancer; chemoresistance; patient-derived organoids; single-cell RNA sequencing, tumor microenvironment; model systems; biomarkers

3. **Accomplishments**

Major goals. As defined in the proposal and SOW, our aims were to obtain local REB and HRPO approval, and then 1. build tissue microarrays (TMAs) for the retrospective cohort; 2. collect, establish organoids models for and characterize the prospective cohort; and 3. validate stroma-inclusive models for potential salvage therapies.

Obtain REB/IRB and HRPO approval. This project was approved by the McGill University Health Centre (MUHC) Research Ethics Board (REB) on 28 May 2021 (subsequent annual renewals approved 31 May 2022 and 9 May 2023). Activities with human subjects conducted therein were approved by the HRPO on 3 Nov 2021.

Goal 1 – Conduct retrospective analyses. Since the retrospective samples required for this part of the project are held within the clinical pathology archives of the institution, the crisis in the healthcare system driven by COVID-19 and staff shortages in the subsequent period have significantly impacted specimen processing. 331 samples have been identified; 232 have been retrieved and are in the digitization, marking and TMA building pipeline.

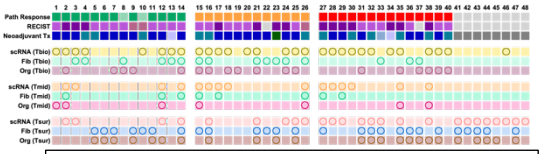

Fig. 1 – Schematic depiction of interim cohort organized by pathological response to neoadjuvant treatment

Goal 2 – Collect and characterize prospective samples.

Sub-Aim 2A. Since receiving final HRPO approval, we have enrolled a total of 36 adenacarcinoma patients into the longitudinal sample collection workflow. Of the enrolled patients (29 male/7 female; average age 67 yrs, range 49-86 yrs), organoids have so far successfully been generated for 14 initial biopsies (Tbio), 5 on-treatment biopsies (Tmid) and 9 resection samples (Tsur). In addition, we have isolated and cultured cancer-associated fibroblasts (CAFs) and tumor-infiltrating lymphocytes (TILs) from 17 and 20 Tbio, 12 and 13 Tmid and 11 and 12 Tsur samples, respectively. For several of these longitudinal cases, only the Tpre sample (or Tpre and Tmid) have been obtained so far and subsequent samples across the timeline remain to be collected. The interim cohort, which includes additional patients with available data, is presented in Fig 1.

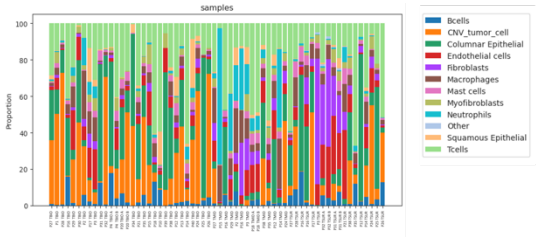

Fig. 2 – Cell type proportions as determined from analysis of scRNAseq data across patients and timepoints.

Sub-Aim 2B. Analyses of scRNA-seq data from pre-, mid- and post--treatment samples revealed that multiple cell types could be identified, and that the proportions of these were variable between patients and across the treatment continuum (Fig. 2). We further investigated specific cell types

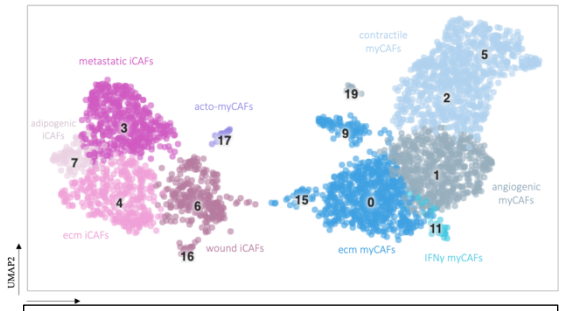

Fig. 3 – UMAP depiction of fibroblast populations in chemonaïve samples. Left, iCAFs; right, myCAFs.

associated with the tumor microenvironment (TME), starting with cancer-associated fibroblasts (CAFs). Within pre-treatment (chemonaïve biopsy) samples, we were able to segregate these into inflammatory (iCAF) and myofibroblast (myCAF) populations (Fig. 3). In the chemonaïve (pre-treatment) population, we then sought transcripts within these subpopulations with expression levels correlated to downstream response to treatment. In iCAFs, this identified CCL20, high expression of which in iCAFs was linked to worse pathological outcome (consistent with reported pro-inflammatory roles in breast and overian cancer) and CHRDL1, a BMP anatagonist with elevated expression in good pathological cases (Fig 4). Similarly, we divided patients into good- and poor-clinical response (RECIST) groups, and identifed ISG20 as differentially expressed – initial experiments indicate that this observations is conserved at the protein level. We then similarly analyzed the fibroblast-specific transcriptional data across the treatmnt continuum (at pre-vs. mid- vs. post-treatment timpepoints) to identify genes where changes in expression patterns displayed significant correlations with response, and would thus be potentially linked to acquisition of chemoresistance. This identified the cohesin component STAG1 (whose expression decreases over the treatment time-course in poor- vs. good response cases), as well as PLCG2 (Fig. 6) – this transcript, expression of which rises in good- but not poor-response cases during treatment, has previously been identified as positively correlated with anti-tumor immune activation in sarcoma. In addition, we further investigated expression of the previously identified potential biomarker COL10A1, confirming its link to overall survival and expression within a restricted fibroblast population (Fig. 7).

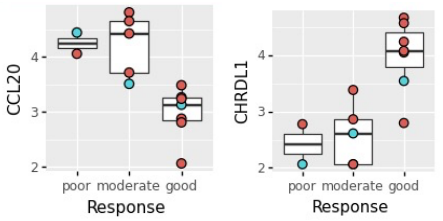

Fig. 4 – Expression of outcome-linked genes in iCAFS of pre-treatment patient samples.

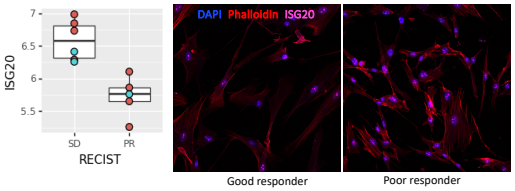

Fig. 5 – Left, expression of ISG20 in pre-treatment fibroblasts from stable disease vs partial response cases. Right, immunofluorescence assys for IG20 is good- vs. poor-responder fibroblasts

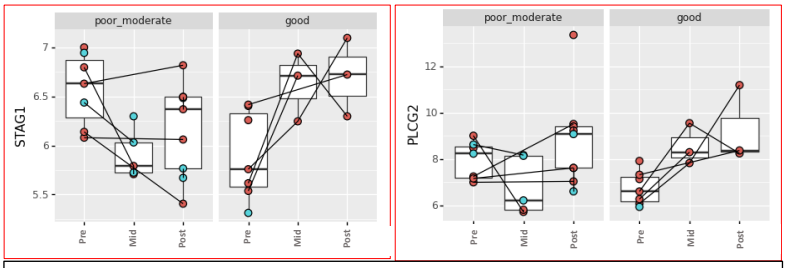

Fig.6– Longitudinal expression of response-linked genes in fibroblasts.

Goal 3 – Validate TME-inclusive screening strategies and model systems. We have developed coculture models for tumor organoids and isolated fibroblasts and demonstrated that these can be used to recapitulate patient response. We have further developed theEsophagus-on-a-chip (EOAC; Emulate) microfluidic system, which permits both integration of TME elements reproduction of flow and mechanical forces. Testing of chemotherapy agents in this model demonstrates that samples from good and poor responders recapitulate the effects observed in patients. (Fig. 8).

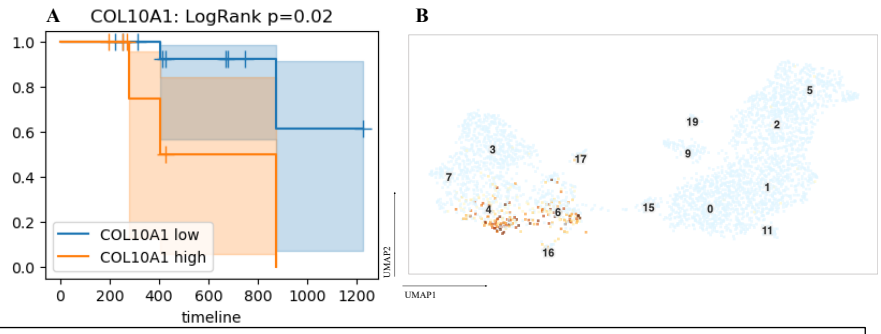

Fig. 7 – A, Kaplan-Meyer curve for populations with high or low fibroblast expression of COL10A1. B, UMAP for COL10A1-expressing cells (brown) across fibroblasts

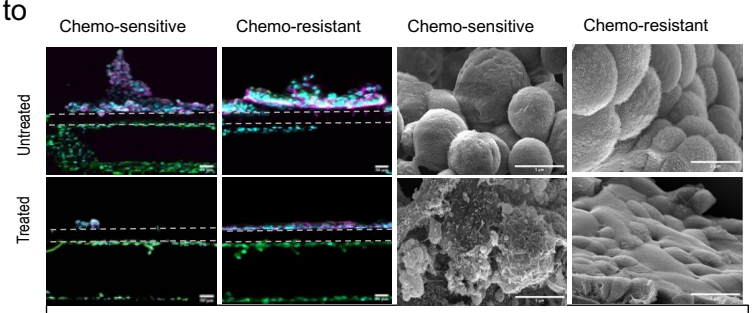

Fig. 8 – Images of tumor (upper channel) and fibroblast (lower channel) cells in esophagus-chip system before (upper row) and after (lower row) treatment. Left images, immunofluorescence imaging (epithelial marker CK7, magenta; fibroblast marker vimentin, green); right, scanning electron microscopy of tumor cells.

4. **Impact.** At this time, it remains too early for any significant contributions or changes in practice to have come about as a result of this project in the principal or other disciplines, or on society in general. Thus, there is nothing to report under this section. No technology transfer has been realized to date.

5. **Changes/Problems**

Retrieval of archival samples for retrospective studies were previously impacted by personnel shortages during the COVID-19 health crisis – there remain some ongoing issues since the backlog of clinical work generated during that time is still being addressed.

There have been no significant changes in use of human subjects; changes in care are not envisaged in the current scope of this project.

No vertebrate animals are used in this study.

No changes in use of biohazards or select agents have occurred.

6. Products

No publications, books, conference papers or presentations have resulted from this work to date.

No websites have been established to disseminate the results of research activities.

No novel technologies or techniques have been created, and no inventions, patent applications or licenses have resulted to date.

7. Participants & Other Collaborating Organizations

Name: *Lorenzo Ferri*
Project Role: *PI*
Researcher Identifier (e.g. ORCID ID): *n/a*
Nearest person month worked: *2*
Contribution to Project: *Dr. Ferri directed experiments, led the project and selected participants*
Funding Support: *No salary from this source*

Name: *Veena Sangwan*
Project Role: *Co-investigator*
Researcher Identifier (e.g. ORCID ID): *n/a*
Nearest person month worked: *1*
Contribution to Project: *Dr. Sangwan supervised personnel and directed experiments*
Funding Support: *No salary from this source*

Name: *Swneke Bailey*
Project Role: *Co-investigator*
Researcher Identifier (e.g. ORCID ID): *n/a*
Nearest person month worked: *1*
Contribution to Project: *Dr. Bailey directed computational analysis*
Funding Support: *No salary from this source*

Name: *Jonathan Cools-Lartigue*
Project Role: *Co-investigator*
Researcher Identifier (e.g. ORCID ID): *n/a*
Nearest person month worked: *0.25*
Contribution to Project: *Dr. Cools-Lartigue assisted with patient selection and provided clinical insight*
Funding Support: *No salary from this source*

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Project Role: *Co-investigator*
Researcher Identifier (e.g. ORCID ID): *n/a*
Nearest person month worked: *0.25*
Contribution to Project: *Dr. Park provided guidance with analysis of the TME*
Funding Support: *No salary from this source*

Name: *Sui Huang*
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Nearest person month worked: *0.25*
Contribution to Project: *Dr. Huang directed analysis of computational data*
Funding Support: *No salary from this source*

Name: *Kulsum Tai*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): n/a
Nearest person month worked: *4*
Contribution to Project: *Ms. Tai conducted work in the area of fibroblast analysis*
Funding Support: *CDMRP and an FRQ-S studentship award*

Name: *Ruo Yu Ma*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): n/a
Nearest person month worked: *2*
Contribution to Project: *Mr. Ma conducted experiments in drug screening*
Funding Support: *CDMRP and institutional/Foundation funding.*

Name: *Sanjima Pal*
Project Role: *Post-Doctoral Fellow*
Researcher Identifier (e.g. ORCID ID): n/a
Nearest person month worked: *5*
Contribution to Project: *Dr. Pal worked on the development and optimization of the EOAC system*
Funding Support: *CDMRP and institutional/Foundation funding.*

Name: *Betty Giannias*
Project Role: *Laboratory Technician*
Researcher Identifier (e.g. ORCID ID): n/a
Nearest person month worked: *8*
Contribution to Project: *Ms. Giannias conducted experiments and processed samples*
Funding Support: *CDMRP and institutional/Foundation funding.*

Name: *Vanessa Brien*
Project Role: *Summer student*
Researcher Identifier (e.g. ORCID ID): n/a
Nearest person month worked: *3*
Contribution to Project: *Ms. Brien collected clinical data and follow-up for patients in study cohort.*
Funding Support: *CDMRP*

8. **Special Reporting Requirements** – n/a

9. **Appendice** – n/a