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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 to 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 and TME components), innovative coculture platforms and high-throughput drug screens.

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

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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 half of those who do respond develop resistance later. Given the historical paucity of readily identifiable 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 chemotherapy-resistant cases. Our overall goal is to investigate and position TME-directed 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 in the initial year of this project 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. Subsequently, 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 current personnel crises in the healthcare system driven by COVID-19 have significantly impacted specimen retrieval. Samples have been selected and retrieval is pending.

Goal 2 – Collect and characterize prospective samples. Since receiving final HRPO approval, we have enrolled a total of 19 patients into the longitudinal sample collection workflow. Of these, 3 were classified as squamous cell carcinomas upon review of initial diagnostic biopsy material while 1 was stratified for combined chemo- and radiotherapy in the neoadjuvant treatment setting; thus, 4 cases were excluded. Of the 15 enrolled patients (10 male/5 female; average age 67 +/- 11 years), organoids have so far successfully been generated for 6 initial biopsies (Tbio), 3 on-treatment biopsies (Tmid) and 4 resection samples (Tsur). Initial analyses of scRNA-seq data from pre- and mid-treatment samples revealed that

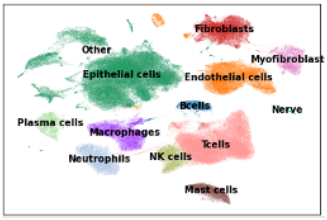


Fig. 1 – entire dataset with clusters identified by inferred cell type

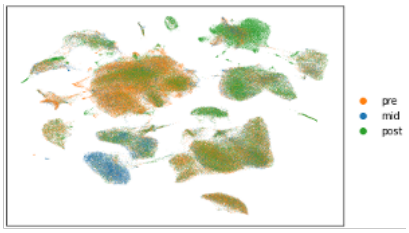


Fig. 2 – entire dataset colored by point in treatment continuum

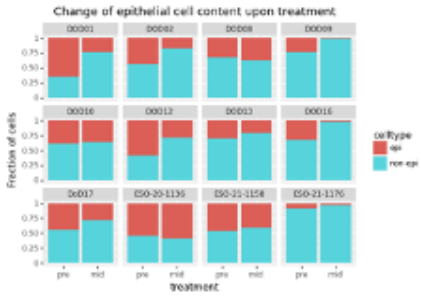
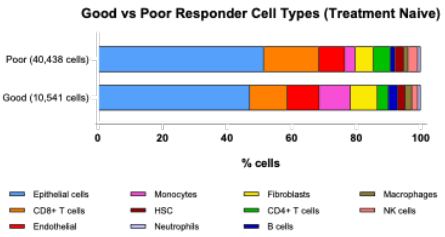
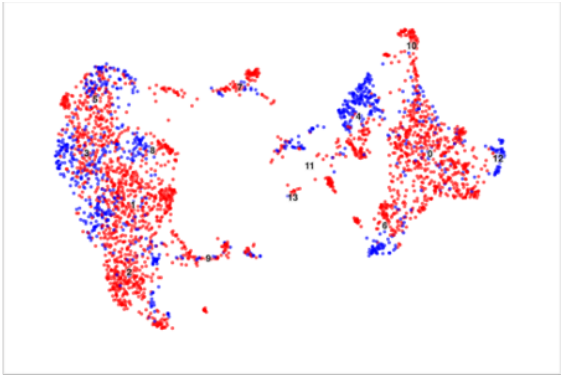


Fig. 3 – impact of treatment on relative proportions of epithelial and stromal cells

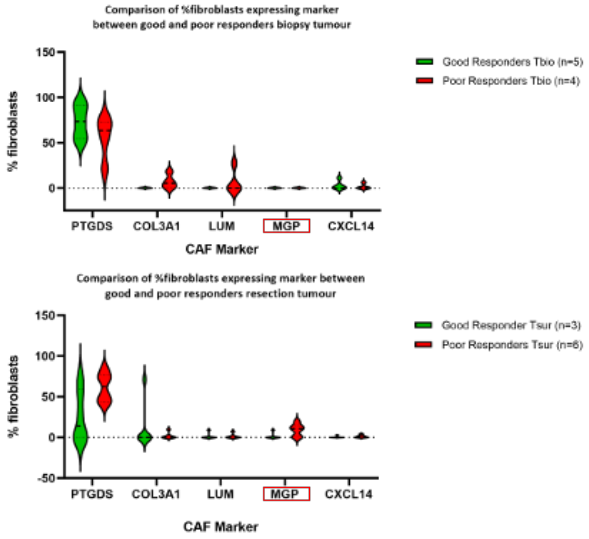


Fig, 4. Cell type proportions prior to treatment in good and poor responder samples

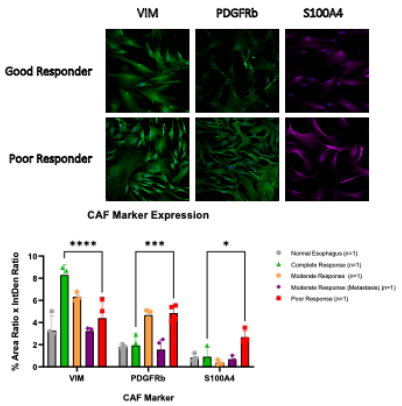
multiple cell types could be distinguished (Fig 1), and that changes could be observed across the treatment continuum (Fig. 2). Specifically, the epithelial cell component decreased after treatment (as expected), while neutrophil presence peaked in the mid-treatment samples and B cells were most highly represented in the post-treatment setting. Importantly, we observed that both the starting relative proportions of epithelial (tumor) and stromal cell and the change in those proportions at the Tmid timepoint is highly variable between individual patients (Fig. 3). We then looked specifically within our scRNA-seq dataset of only pre-treatment sample to assess potential differences in epithelial and TME cells between poor and good responders, and observed that cell type proportions appeared to vary between these two populations (Fig. 4). Looking specifically at one cell type, fibroblasts, we identifed subclusters within pre-treatment samples that were predominantly associated with good or poor response (Fig. 5), further supporting the hypothesis that TME features are associated with chemoresistance. Looking at specific fibroblast-associated transcripts in the context of pre- and post-therapy data, we identified a set that vary between good and poor responders in the pre- and post-treatment settings (Fig. 6). Interestingly, matrix Gla protein (MGP), the elevated expression of which has been linked to poor outomce in several tumor types, increases in poor- but not good-response samples after treatment, suggesting a potential role in chemoresistance. We further investigated the expression of several of these



Fig, 5. Fibroblast subclusters associated with poor or good response



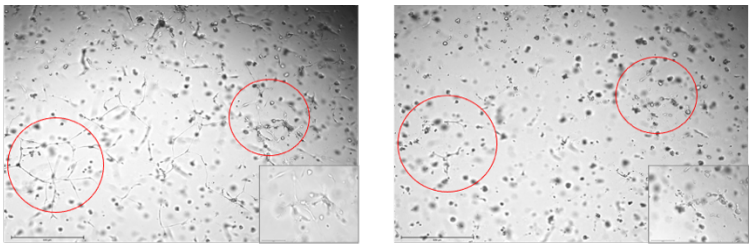
Fig, 6. Fibroblast transcripts that vary between good and poor responders in the pre- (above) and post-(below) treatment settings



Fig, 7. Expression of markers in fibroblast cultures from goo- and poor-response cases

markers in cultures of isolated fibroblasts from poor- and good-response patients (Fig. 7) and confirmed their differetial expression. We will continue to enrol additional patients, collect samples through their treatment trajectory and generate organoids as well as isolating matched fibrobalsts and tumor-infiltrating lymphocytes (TILs), as well as generating genomic and transcriptomic data to add to our existing dataset for further analyses.

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 (Fig. 8). As a more physiologically relevant but lower-throughput model, we have also continued optimization of the Esophagus-on-a-chip (EOAC) 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, 9).



Fig, 8. Tumor organoid-fibroblast cocultures recapitulate response observed in patient (good responder)

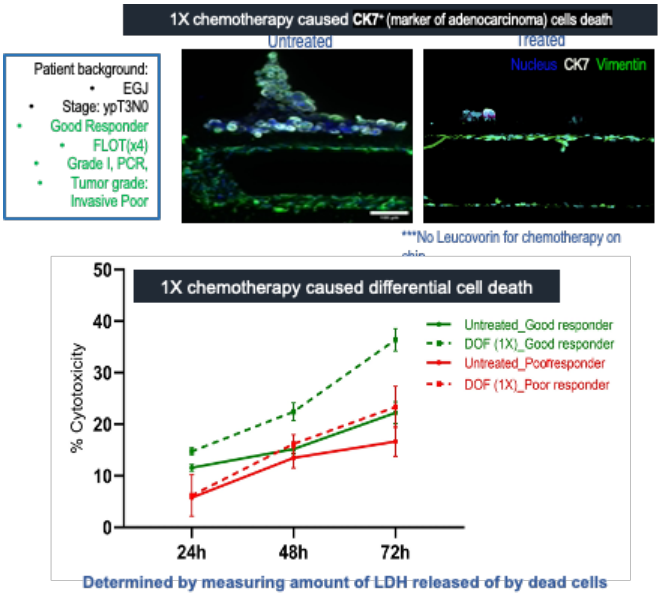


Fig. 9 Tumor cell/fibroblast EOAC models recapitulate clinically observed responses

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; also, no technology transfer has been realized yet.

5. **Changes/Problems**

Retrieval of archival samples for retrospective studies was impacted by personnel shortages during the COVID-19 health crisis. We identified a source of sample attrition in cases where pathology review of the diagnostic biopsy sample from enrolled cases revealed squamous cell carcinoma, rather than our target histology of adenocarcinoma.

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: *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: *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*

Name: *Morag Park*
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*
Project Role: *Co-investigator*
Researcher Identifier (e.g. ORCID ID): *n/a*
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 areas of fibroblast analysis/*
Funding Support: *Ms. Tai is partially supported from this funding source, and is also supported by 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: *Mr. Ma is partially (81%) supported from this funding source.*

Name: *Wo Tan Zeng*
Project Role: *Research Assistant*
Researcher Identifier (e.g. ORCID ID): *n/a*
Nearest person month worked: *3*
Contribution to Project: *Ms. Zeng processed samples, prepared single cells and generated organoids*

Funding Support: *Ms. Zeng (new hire, replaces Ms Wang below) is fully supported from this funding source.*

Name: *Yun Wang*
Project Role: *Research Assistant*
Researcher Identifier (e.g. ORCID ID): *n/a*
Nearest person month worked: *9*
Contribution to Project: *Ms. Wang processed samples, prepared single cells and generated organoids*
Funding Support: *Ms. Wang was fully supported from this funding source.*

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: *Dr. Pal is partially (40%) supported from this funding source*

Name: *Betty Giannias*
Project Role: *Laboratory Technician*
Researcher Identifier (e.g. ORCID ID): *n/a*
Nearest person month worked: *12*
Contribution to Project: *Ms. Giannias conducted experiments and processed samples*
Funding Support: *Ms. Giannias is partially (33%) supported from this funding source*

8. Special Reporting Requirements

n/a

9. Appendices

n/a