Abbreviated Title: Esophageal Metabolomics

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Title: Metabolomic and BH3 Profiling of Esophageal Cancers: Identification of Novel Assessment Methods of Treatment Response for Precision Therapy

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F. Studying, interpreting, or analyzing de-identified data or specimens for research purposes

G. Some/all research activities performed outside NIH

Investigational Agents:

NA

Commercial Agents:

NA

PRÉCIS

Background:

- The incidence of esophageal cancer continues to increase with an estimated 16,900 new cases and 15,700 deaths in 2016.[1] Esophageal adenocarcinoma (EAC) is the dominant histology in the United States and accounts for the rising incidence; the incidence of esophageal squamous cell cancer (ESCC) remains stable.
- Neoadjuvant chemoradiotherapy (nCRT) followed by esophagectomy is now a standard approach for locally advanced, operable esophageal cancer.
- A survival advantage compared to surgery alone was demonstrated in the phase III Chemo Radiotherapy for Oesophageal cancer followed by Surgery Study (CROSS) trial.[2]
- Patients who experience a pathological complete response (pCR) following neoadjuvant therapy are most likely to have long-term survival.[3, 4]
- Presently, accurate assessment of pathologic response requires esophagectomy. Positron-emissions tomography (FDG-PET) and endoscopic evaluation with biopsies fail to detect cancer in a significant percentage of patients with residual disease following neoadjuvant therapy.[5]
- Currently there are no validated tissue or serologic biomarkers which can be used to guide surgical management of esophageal cancer patients based on response to nCRT.

Primary Objective:

- To determine whether a metabolomic signature in tumor, blood, or urine or whether BH3 profiling of pre-neoadjuvant tumor biopsies correlates with the outcome of pathological complete response after neoadjuvant chemoradiotherapy for patients with esophageal adenocarcinoma or squamous cell carcinoma.

Eligibility:

- Patients with locally-advanced, histologically confirmed EAC or ESCC who are candidates for nCRT and esophagectomy.

Design:

- Patients will receive standard of care nCRT either at the NCI or at referring institutions.
- Specimens of plasma, urine, and esophageal tumor with matched normal esophagus will be obtained before neoadjuvant therapy for metabolomic profiling and BH3 profiling. Blood, urine, normal esophagus, and tumor (if present) will be obtained after neoadjuvant therapy.

- Patients will undergo an esophagectomy as a robotically-assisted, minimally-invasive esophagectomy (RAMIE) or a traditional open approach for contraindications to minimally-invasive approaches or based on institutional expertise.
- Analysis will be performed to determine if pCR after CRT correlates with pretreatment metabolomic signatures or BH3 profiling in tumor, blood or urine.
- Patients with EAC and ESCC will be evaluated independently.
- The accrual ceiling will be set to 120 patients for the entire study - 80 patients for EAC and 40 patients for ESCC to allow for unevaluable patients. The accrual is expected to be completed in 4 years.

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1. INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- To determine whether a metabolomic signature in tumor, blood, or urine or whether BH3 profiling of pre-neoadjuvant tumor biopsy correlates with the outcome of pathological complete response after neoadjuvant chemoradiotherapy for patients with esophageal adenocarcinoma or squamous cell carcinoma.

1.1.2 Secondary Objectives

- To evaluate metabolomic profiles and Bcl-2 homology domain-3 (BH-3) profiling in resectable esophageal adenocarcinomas (EAC) and esophageal squamous cell carcinoma (ESCC) treated with neoadjuvant chemoradiotherapy (nCRT).
- To identify whether metabolomic signatures or BH3 profiling in tumor, blood, or urine of EAC and ESCC patients correlate with major responses (Mandard score of 1 and 2) versus minimal response (Mandard score 3-5).
- To examine if tumor and systemic metabolomic signatures correlate with disease-free survival (DFS) or Overall survival (OS) in EAC and ESCC patients undergoing nCRT and esophagectomy.
- To explore whether the specific p53 mutational status correlates to the metabolomic profiles.

1.2 BACKGROUND AND RATIONALE

Esophageal adenocarcinomas (EAC) and esophageal squamous cell cancers (ESCC) accounted for approximately 16,900 cancer cases and 15,700 cancer-related deaths in 2016.[1] EAC is the dominant histology of esophageal cancer in the United States and accounts for the rising incidence. The incidence of ESCC remains stable.[6] Neoadjuvant chemoradiotherapy (nCRT) followed by esophagectomy is now a standard treatment for locally-advanced esophageal cancers for both histologies. A survival advantage compared to surgery alone was demonstrated in the phase III CROSS trial (**Figure 1**). Patients who underwent nCRT followed by esophagectomy had a 47% five-year actuarial survival whereas patients who underwent surgery alone had a 34% five-year actuarial survival.

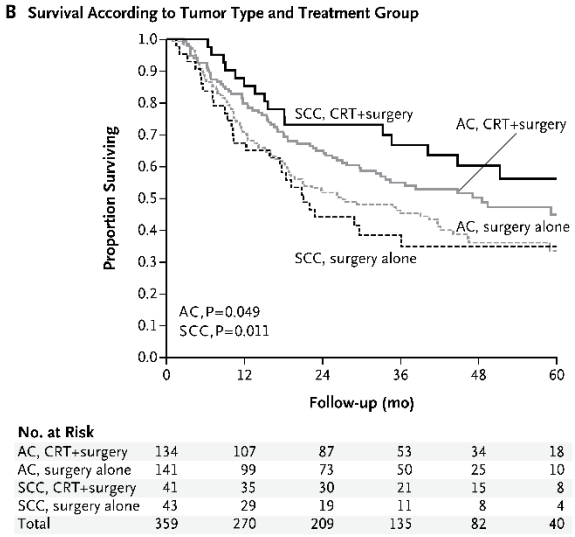


Figure 1: CROSS Trial shows survival advantage with nCRT for both EAC and ESCC. The advantage is more robust for ESCC.

For both histologies, patients who have a pathological complete response (pCR) are most likely to experience long-term survival (pCR is equivalent to stage 0 in figure 2) (**Figure 2**).[3, 4, 7-9] pCR occurs in 17-27% of patients with EAC, whereas, pCR occurs in 40-64% of patients with ESCC.[5, 10-15] An additional group of patients who have a favorable outcomes have a near-pCR with <10% viable tumor cells in the primary esophageal tumor. This response is defined based on the Mandard Score[16] as major response with no viable tumor (grade 1) and <10% viable tumor (grade 2) versus non-major response of >10% viable tumor (grade 3-5) as assessed by final pathology. (**Figure 3**). This group with <10% viable tumor (Mandard 2) constitutes an additional 10-15% of patients who have overall survivals similar to patients with pCR.[17]

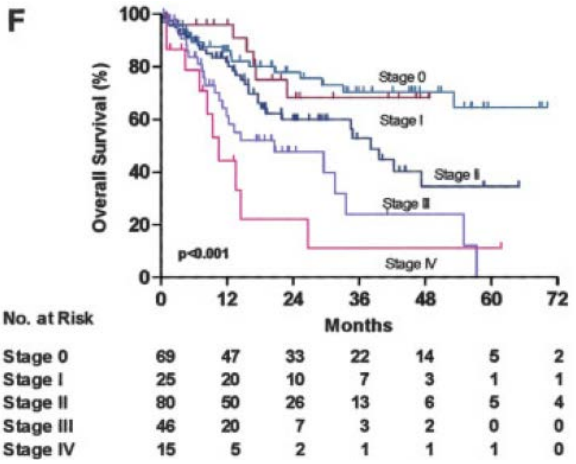


Figure 2: Overall survival based on pathological staging after neoadjuvant therapy. Patients who achieve pathological major responses of stage 0 (equivalent to pCR) and stage I experience a significant survival advantage compared to patients with a minimum response or without a response.

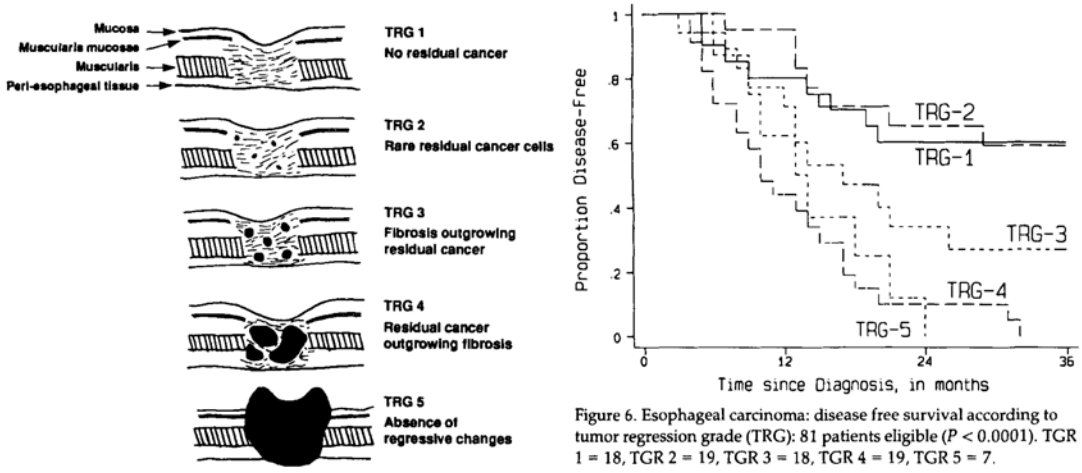


Figure 3: Mandard Score for classification of tumor response after neoadjuvant therapy is associated with disease-free survival in esophageal cancer. A treatment response grade (TRG) of 1 (0% viable tumor cells) or 2 (<10% viable tumor cells) is associated with better survival than TRG of 3-5.

Accurate assessment of response to neoadjuvant therapy will improve care for patients with esophageal cancer.[18] First, prognostic information allows well informed discussions with patients and families regarding options for and expected outcomes after treatment. Second, confirmation of a lack of pathologic response to therapy may prompt discontinuation of ineffective and toxic regimens in favor of other potentially efficacious treatments. Lastly, identification of pCR may enable patients to avoid surgery. The only methods to attempt to identify pCR are 18F-FDG-PET and endoscopic evaluation with biopsies. However, both positron-emission tomography (18F-FDG-PET) and endoscopic evaluation with biopsies fail to reliably predict extent of treatment response, therefore, accurate assessment of pathological response to induction therapy requires esophagectomy.[12, 19, 20]

Resolution of FDG-PET avidity (complete metabolic response: cMR) has been extensively studied after nCRT for EAC and ESCC. In 2016, Heneghan and colleagues reported the sensitivity, specificity, positive predictive value, and negative predictive value of cMR to predict pCR by 18F-FDG-PET to be 56%, 58%, 30%, and 80%, respectively, for EAC and ESCC combined.[5] They reported the number separately for EAC and ESCC and cMR was not associated with pCR for either group. Several other groups have reported strikingly similar results and poor accuracy for FDG-PET after nCRT.[17, 21] Similarly, lack of tumor visualization or biopsy during endoscopy (endoscopic complete response: cER) has been studied.[5, 22] The same group reported the sensitivity, specificity, positive predictive value, and negative predictive value of cER of 41%, 62%, 24%, and 78%, respectively, for EAC and ESCC combined. Again, with EAC or ESCC, cER was not predictive of pCR for either group. They combined cMR and cER to evaluate a complete clinical response (cCR) to determine whether 18F-FDG-PET and endoscopy together were predictive of pCR. The sensitivity, specificity, positive predictive value, and negative predictive value were 32%, 81%, 36%, and 79%, revealing that no method can reliably predict pCR other

than operative resection by esophagectomy. pCR occurred only in 26% of patients with cCR. Another group reported that only 31% had a pCR among patients with cCR.[20] Even with the addition of endoscopic ultrasound (EUS), >25% of esophageal cancers with no clinical signs of residual disease following CRT had residual tumors.[10, 22-24] No method other than 18F-FDG-PET and endoscopy are available to predict response in esophageal cancer, yet neither of these modalities are effective. To date, no tissue or liquid biomarkers which predict treatment response have been validated in esophageal cancer patients. Therefore, we have designed a trial to explore novel methods of predicting response to nCRT through metabolomics and BH3 profiling.

Metabolomics is a method of global detection of small molecule metabolites.[25] It allows analysis of metabolite changes under many conditions including stress, changes in diet, treatment responses, or biological conditions. Increasingly, metabolomics has been used in patients with cancer for detection, prognosis, progression, and treatment response. For example, the metabolite, sarcosine, increases during progression from benign tissue, to localized prostatic tumors, to metastatic disease (**Figure 4**).[26] The association of sarcosine with prostate specific antigen levels has been validated in subsequent trials.[27] In glioblastoma and anaplastic astrocytoma, metabolomics has provided prognostic information. For example, patients with isocitrate dehydrogenase 1 (IDH1) mutations have better overall survival relative to patients with wild-type IDH1 (**Figure 5**).[28] The mutation in this enzyme alters the secreted metabolites which can be detected and characterized by metabolomics. Furthermore, metabolomics has helped guide treatment decisions in patients with chronic myelogenous leukemia; metabolic profiling predicted which patients would response to imatinib.[29] These scenarios are examples of metabolomics that are clinical applicable or close to clinical applicability.

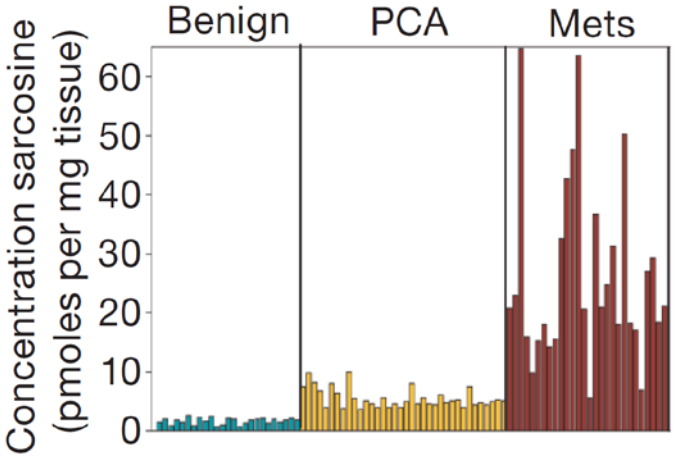


Figure 4: The metabolite, sarcosine, increases with progression from benign prostate to localized prostate cancer to metastatic prostate cancer.

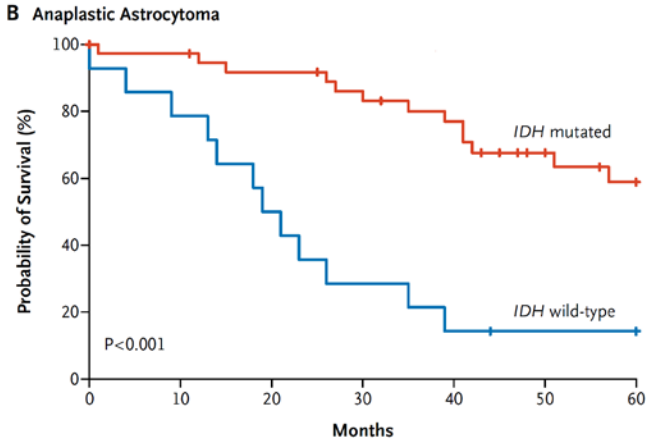


Figure 5: Overall survival in astrocytoma is predicted by mutations affecting the Krebs cycle enzyme, isocitrate dehydrogenase 1 (IDH1).

BH3 profiling is a technique to measure mitochondrial apoptotic threshold of individual tumors to determine whether they are 'primed' for cellular death.[30] Apoptosis is a mechanism of cell death that prevents damaged cells from malignant transformation or progression; the inability for these cells to undergo apoptosis is a well-established hallmark of cancer.[31] The intrinsic pathway of apoptosis induces mitochondrial outer membrane permeabilization (MOMP) which results in a series of events that culminates in cell death via caspase system activation. MOMP is regulated by the Bcl-2 family of proteins that function as pro-apoptotic effectors, anti-apoptotic effectors, the BH3-only activator proteins, and the sensitizer proteins.[32, 33] The interactions of the various members of the Bcl-2 family of proteins occur in the BH3 domain of these proteins. The BH3 domain is about a 20-amino acid alpha helix that enables hetero-dimeric interactions of the Bcl-2 family. Synthetic BH3 oligopeptides can induce apoptosis ex vivo in live cells which enable measuring cellular readiness or 'priming' to undergo apoptosis.[34] BH3 Profiling exploits the interactions of Bcl-2 family proteins in live tumor cells with the synthetic BH3 oligopeptides to quantitatively measure individual patient's tumor susceptibility to cytotoxic therapy.[35]

BH3 Profiling has successfully predicted response to cytotoxic chemotherapy and resistance to targeted therapy in clinical samples and cell culture models of solid tumors.[30] In 16 patients with ovarian adenocarcinoma, the differences in priming measured by BH3 Profiling predicted a significant difference in progression-free survival after treatment with carboplatin and Taxol. Conveniently, this regimen uses the same cytotoxic drugs as the CROSS regimen. In the same manuscript, Montero and colleagues report that BH3 profiling predicted acquired resistance to tyrosine kinase inhibitors (TKI) in the non-small cell lung cancer cell (NSCLC) line, PC9. Parental PC9 cells are gefitinib-sensitive but with chronic exposure, these cells developed resistance via the well-described T790M point mutation (PC9GR). The PC9GR cells were sensitive to the mutant selective EGFR TKI, WZ4002, but developed resistance with chronic exposure to WZ4002. BH3 Profiling successfully predicted the emergence of resistance with chronic exposure of each TKI. These two examples are of a very few examples of BH3 Profiling in solid tumors.

We hypothesize that metabolomics may provide a signature associated with response to nCRT for patients with EAC and ESCC. Additionally, we hypothesize that BH3 profiling will predict pCR in patients treated with nCRT. Patients with EAC and ESCC will be evaluated independently. Tumor, normal esophageal, blood, and urine specimens will be analyzed for metabolic profiles.

Tumor only will be used for BH3 profiling. To test BH3 profiling, at the time of pre-neoadjuvant biopsy for metabolic profiling, part of the tumor sample will be sent to the Ripley laboratory to perform BH3 Profiling on the live cells. This technique will be performed in our laboratory in collaboration with Dr. Letai's laboratory at the Dana Farber Cancer Institute. We will compare the metabolic signature and BH3 profiling of patients who have pCR versus those who do not. Given that patients with a near-pCR (Mandard 2) do similarly well, we will perform a second analysis of major response (Mandard 1-2) versus minimal response (Mandard 3-5). This difference is subtle, but it evaluates patients based on standard methods. The comparison of the Mandard 1-2 will add patients with <10% viable tumor to the pCR group. We believe that characterization of both a metabolic signature and BH3 profiling will guide future management of esophageal cancer.

In addition to defining the metabolomic signature and BH3 profiling, we will evaluation whether the p53 mutational status affects this signature. Evaluation of p53 mutational status is important in esophageal cancer because 50 to 70% of esophageal cancers harbor p53 mutations. p53 mutations not only result in loss of tumor suppressor activity but also convert p53 to a gain-of-function oncogenic driver mutation.[36] Additionally, while wild-type p53 induces apoptosis under conditions of stress by upregulation of mitochondrial death signals, these pathways are not activated with mutant p53.[37] EAC patients with these mutations do not respond well to platinum-based therapy and have worse outcomes after either surgery alone or nCRT.[38, 39] As preclinical data has shown, p53-mutant pancreatic cells and xenografts may be more susceptible to metabolic inhibitors.[40] Therefore, determining whether the p53 mutational status alters the metabolic signature will be an secondary aim of this protocol. This analysis may help determine whether to pursue different therapeutic strategies for EAC based on the p53 mutational status.

The purpose of this study is to determine whether a metabolomic signature or BH3 profiling can predict pCR in patients with esophageal cancer. Predicting pCR will provide prognostic information, confirm lack of therapeutic benefit, and avoid surgery with pCR. In preclinical models, metabolomics is being developed for future therapeutics in addition to biomarker discovery.[41, 42],[43, 44] Presently, limited data are available pertaining to metabolic pathways in esophageal cancers. In addition to the assessment of treatment response in this trial, we anticipate that characterization of the esophageal metabolome may serve as a basis for precision-based, personalized strategies for future clinical trials.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have histologically confirmed EAC or ESCC.

2.1.1.2 Disease should be deemed resectable by pre-operative CT and/or PET scans and the patient should be operable based on surgeon assessment.

2.1.1.3 Patients willing to complete nCRT per standard of care followed by esophagectomy. Patients will be treated under protocol 04-C0165.

2.1.1.4 ≥18 years of age.

2.1.1.5 Able to understand and sign the Informed Consent Document.

2.1.1.6 ECOG performance status ≤2 (see **Appendix A**).

2.1.1.7 Patients must have organ and marrow function that is not prohibitive of surgical resection as defined below:

- leukocytes ≥3,000/mcL
- absolute neutrophil count ≥1,500/mcL
- platelets ≥50,000/mcL

2.1.1.8 nCRT used in this study is potentially dangerous for developing human fetus. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration and 6 months' post chemoradiotherapy. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

2.1.1.9 Women must have a negative urine pregnancy test OR be post-menopausal for at least 2 years OR patient has had a hysterectomy.

2.1.2 Exclusion Criteria

2.1.2.1 Patients in which nCRT followed by surgery is not the appropriate management:

- Early stage disease that requires local therapy without CRT.
- Patients with metastatic disease.

2.1.2.2 Patients in which biopsy prior to starting nCRT is not obtainable.

2.1.2.3 Patients who previously received neoadjuvant chemotherapy

2.1.2.4 Concomitant medical problems in the opinion of physician that would place the patient at unacceptable risk for a major surgical procedure.

2.1.2.5 Active systemic infections, coagulation disorders or other major medical illnesses of the cardiovascular, respiratory or immune system, myocardial infarction, heart failure, hepatic disease that prohibits administration of neoadjuvant therapy or surgery.

2.1.2.6 Women who are pregnant or breastfeeding because of the potentially dangerous effects of the chemotherapy on the fetus or infant.

2.1.2.7 Patients with a diagnosis of another malignancy that is either active or in remission less than five years. Basal cell and squamous cell carcinoma of the skin are not contraindications to this protocol

2.2 RECRUITMENT STRATEGIES

Subjects will be referred from institutions in the local area from surgeons and gastroenterologists who already refer patients.

Publication of this protocol in Trials magazine in order to be searchable on PubMed (Introduction with inclusion and exclusion criteria of this protocol).

Searchable on Clinicaltrials.gov, listed on Thoracic and GI Oncology website, listed on CCR webpage for the PI (Dr. Ripley).

2.3 SCREENING EVALUATION

Patients will undergo the following screening evaluations which may be performed within 45 days prior to enrollment:

2.3.1 Complete history and physical examination including vital signs, height, weight, ECOG assessment.

2.3.2 CT or PET-CT scan of chest, abdomen and pelvis.

2.3.3 Histologic or cytologic confirmation (at any time point prior to enrolment).. (If patient does not have available pathology report or samples from previous biopsies, EGD with biopsy will be performed)

2.3.4 Laboratory evaluations:

- CBC with platelets
- Biochemical profile: (Sodium (Na), Potassium (K), Chloride (Cl), total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), albumin and pre-albumin.
- PT/PTT & INR, CRP.
- Beta HCG for women of child bearing potential

2.4 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

2.5 TREATMENT ASSIGNMENT PROCEDURES

Cohorts

Number	Name	Description
1	Cohort 1	Subjects with locally-advanced, histologically confirmed esophageal adenocarcinoma who are candidates for nCRT and esophagectomy
2	Cohort 2	Subjects with locally-advanced, histologically confirmed esophageal squamous cell cancer who are candidates for nCRT and esophagectomy

Arms

Number	Name	Description
1	Arm 1	Standard of care nCRT and esophagectomy.

Arm Assignment

Patients in Cohorts 1 and 2 will be directly assigned to Arm 1.

2.6 BASELINE EVALUATION

In the vast majority of patients, the screening and baseline evaluations will be the same evaluation given that patients are enrolled prior to therapy for esophageal cancer. The pre-nCRT biopsy by endoscopy is the critical step to obtain tissue. Patients will be enrolled prior to treatment, then they will usually return to referring institutions for standard of care nCRT. After that treatment, they will return to the NIH for esophagectomy. For the occasional patients who undergo nCRT at the NIH, they will be enrolled on protocol 04C0165.

To be performed within 45 days prior to initiation of nCRT (these tests will not need to be repeated if they were done at screening within the appropriate timeframe):

2.6.1 Nutritional Assessment: All patients seen at the NIH will receive a nutrition evaluation by a Clinical Center dietitian at baseline and follow-up consultation at regular intervals. Nutrition assessment for patients treated at the NCI will be under the direction of the AI, Rachael Lopez, MPH, RD, CSO (rachael.lopez@nih.gov; Office: (301) 594-3084).

Another member of the Clinical Center dietitian staff will evaluate and monitor when Ms. Lopez is unavailable.

The first nutritional assessment is usually for baseline nutrition status and to optimize nutrition status prior to neoadjuvant treatment as needed. The pre-esophagectomy assessment would start the education on the post-op diet and provide nutrition supplements pre-operatively if warranted. Follow-up after surgery typically takes the most time to help patients minimize weight loss and to help troubleshoot diet intolerances and GI symptoms as the patients adjust to the post-operative diet.

2.6.2 Complete history and physical examination including vital signs, weight and ECOG assessment.

2.6.3 Concomitant medications

2.6.4 CT and/or PET-CT scan of chest, abdomen and pelvis.

2.6.5 Pulmonary Function Tests (PFT's) if indicated.

2.6.6 Esophagogastroduodenoscopy (EGD) with tumor and normal esophagus biopsies. Tumor biopsy and normal esophageal biopsy will be performed as part of the routine evaluation by EGD. Often, patients have received one EGD at the time of initial diagnosis, however, repeating the EGD prior to neoadjuvant therapy is routinely performed for the surgeon to assess tumor location, proximal esophagus, gastric involvement, and confirmation of

disease. These assessments are often not possible after nCRT. Standard biopsy forceps in the EGD endoscope will be used to obtain the tissue biopsy.

2.6.7 Endoscopic Ultrasound (if indicated)

2.6.8 Laboratory evaluations

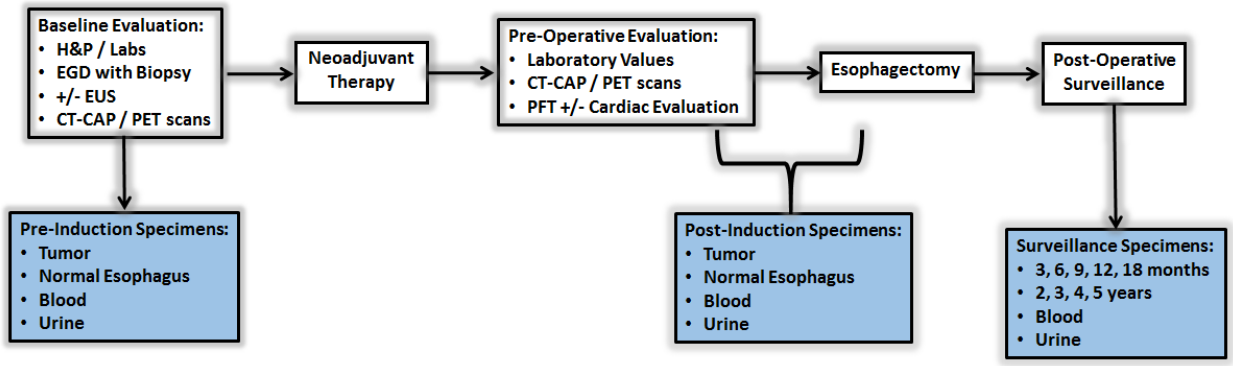
- CBC with platelets
- Biochemical profile: (Sodium (Na), Potassium (K), Chloride (Cl), total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), albumin, pre-albumin
- PT/PTT & INR, CRP.
- Beta HCG for women of child bearing potential (within 28 days)
- Correlative studies performed at baseline are specified in Section **5**

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

Patients with resectable EAC or ESCC who are candidates for nCRT followed by surgery will be evaluated by associate investigators in coordination with the Principal Investigator for eligibility.

Cohort 1: 80 patients with EAC.



Cohort 2: 40 patients with ESCC.

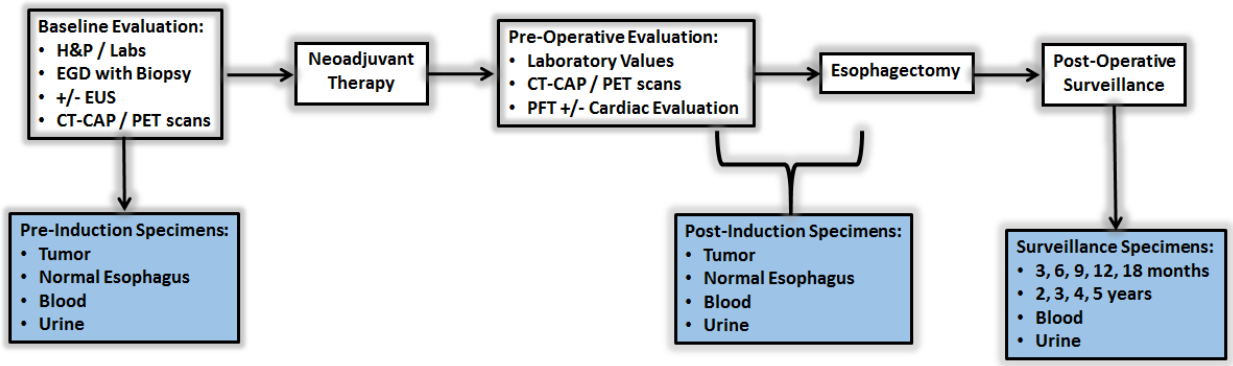


Figure 6: NCI schema for clinical treatment pathway of patients with resectable EAC or ESCC. Patients with EAC and ESCC will be analyzed independently.

3.2 NEOADJUVANT THERAPY

Patients who undergo nCRT at the NCI on protocol 04C0165, they will be treated based on the CROSS regimen with carboplatin and paclitaxel for two cycles with 40.4 Gy of radiotherapy which follows NCCN guidelines.

Figure 6). Patients may receive chemoradiotherapy from their local physician but must come to the NCI for surgery once the neoadjuvant therapy has completed.

3.2.1 Paclitaxel

Paclitaxel 50 mg/m^2 should be given by intravenous infusion on days 1, 8, 15, 22 and 29.

At hour 0, the total calculated dose of paclitaxel, diluted in 500 ml of 0.9% NaCl will be infused over one hour.

Prior to Paclitaxel infusion, 100 ml 0.9% NaCl will be infused over 0.5 h, followed by an infusion of 8 mg Ondansetron or its equivalent diluted in 100 ml 0.9% NaCl over 1.5 hour.

- Premedication:

All patients receiving Paclitaxel should receive premedication 30 minutes prior to the start of infusion according to the following schedule:

Premedication	Dosage	Timeframe
Dexamethasone	10 mg IV	0.5 hour prior to Paclitaxel
Diphenhydramine	12.5-25 mg po	0.5 hour prior to Paclitaxel
Ranitidine (Zantac)	50 mg IV	0.5 hour prior to Paclitaxel

3.2.2 Carboplatin

Carboplatin AUC = 2 should be given by intravenous infusion on days 1, 8, 15, 22 and 29.

The total calculated dose of Carboplatin, diluted in 500 ml 5% Dextrose injection should be infused over one hour (doses Carboplatin > 250 mg should be dissolved in 1000 ml 5% Dextrose Injection).

The absolute dose of Carboplatin should be calculated for the target AUC = 2 according to the following formula:

- The absolute dose of Carboplatin = [target AUC] x (GFR + 25).
- Formula GFR = [((140 – age) x 1.23 x body weight) / serum creatinine X (0.85 (female) or 1.00 (male))]

3.2.3 Infusion Scheme

Paclitaxel/Carboplatin Infusion Scheme	
-1-0.5 hrs	Premedications and Ondansetron (or its equivalent) 8 mg in 100 ml 0.9% NaCl
0.00 +/- 2 hrs	…… mg Paclitaxel in 0.9% NaCl 500ml (PVC free)
1.00 +/- 2 hrs	0.9% NaCl 100 ml
2.00 +/- 2 hrs	…… mg Carboplatin in 500 ml 5% Dextrose Injection in 1 hour

3.2.4 Patient monitoring

Some patients may experience asymptomatic bradycardia during the paclitaxel infusion. In addition, hypersensitivity reactions are possible and generally occur within the first few minutes of initiating the infusion. For these reasons, the recommendation is for constant supervision with the vital signs monitoring every fifteen minutes during paclitaxel administration. Thereafter, patients may be observed and heart rate and blood pressure checked if necessary, according to clinical symptoms.

3.3 RADIATION THERAPY GUIDELINES

Patients will receive standard of care neoadjuvant therapy either at the NCI or, more commonly, at referring institutions. Appropriate institutional guidelines will be followed.

For those patients receiving therapy at the NCI the following administration guidelines will apply.

3.3.1 Fractionation schedule

A total dose of 40.4 Gy will be given in 23 fractions of 1.8 Gy, 5 fractions per week, starting the first day of the first cycle of chemotherapy. All patients will be radiated by external beam radiation, using 3-D conformal radiation technique.

The Gross Tumor Volume (GTV) is defined by the primary tumor and any enlarged regional lymph nodes, and will be drawn on each relevant CT slice. The GTV will be determined using all available information (physical examination, endoscopy, EUS, CT-thorax/abdomen, PET).

The Planning Target Volume (PTV) will provide a proximal and distal margin of 4 cm, in case of tumor extension into the stomach, a distal margin of 3 cm will be chosen. A 1.5 cm radial margin around the GTV will be provided to include the area of subclinical involvement around the GTV and to compensate for tumor motion and set-up variations.

Both lungs will be contoured. The heart will be contoured on all slices; its cranial border will include the infundibulum of the right ventricle and the apex of both atria, and will exclude the great vessels as much as possible. The caudal border will be defined as the lowest part of the left

ventricle's inferior wall that is distinguishable from the liver. The spinal canal will be contoured and taken to represent the spinal cord.

3.3.2 Normal tissue tolerance

Dose-volume histogram (DVH) of both lungs, the heart and spinal cord will be obtained for all patients.

3.3.3 External beam equipment

Radiation therapy will be delivered with megavoltage equipment with photon energies of equal to or greater than 6 MV. 3D CRT, IMRT, and VMAT are allowed.

3.3.4 Dose specification

The prescription dose will be specified at the ICRU 50/62 reference point, which will be the isocenter for most patients. The daily prescription dose will be 1.8 Gy at the ICRU reference point and the 95% isodose must encompass the entire planning target volume (PTV). The maximum to the PTV must not exceed the prescription dose by >7%. Tissue density inhomogeneity correction will be used.

Modifications to the target volume, normal tissue dose limits, fractionation, and total dose may be made at the discretion of the treating radiation oncologist as clinically appropriate.

3.4 SURGICAL GUIDELINES

3.4.1 Pre-op Assessment

After neoadjuvant therapy, patients will be evaluated by standard pre-op assessment which may include:

3.4.1.1 Pulmonary function tests (PFT's) if indicated

3.4.1.2 Cardiac evaluation as indicated; EKG if clinically indicated

3.4.1.3 Complete history and physical examination including vital signs, weight and ECOG assessment.

3.4.1.4 CT, 18F-FDG-PET, or 18F-FDG-PET-CT scan of chest, abdomen and pelvis

3.4.1.5 Laboratory evaluations:

- CBC with platelets
- Biochemical profile: (Sodium (Na), Potassium (K), Chloride (Cl), total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), albumin and pre-albumin
- PT/PTT & INR, CRP.
- Beta HCG for women of child bearing potential

3.4.2 Clinical Assessment for Fitness for Surgery and Pre-Operative Patient Management

Patients will receive standard preoperative care as appropriate to the planned surgical intervention and the patient's underlying health status which may include:

- The day prior to surgery: Incentive spirometry, appropriate bowel preparation regimen, and hydration if needed

- Patient Management in the Operating Room
- Patients will receive perioperative antibiotics and the first dose will be administered prior to incision.
- Epidural catheters will be given in all patients unless patient unable or unwilling to receive. Decisions will be made by the anesthesiologist and the operating surgeon in full collaboration.
- Sequential compression devices will be "on" before induction of anesthesia.

3.4.3 Surgical Procedure

Patients will undergo a robotically-assisted, minimally-invasive esophagectomy (RAMIE) if feasible at the NCI. For those whom a minimally-invasive procedure is contraindicated or for institutions that do not perform minimally-invasive procedures, a traditional open approach will be performed. Tumors will be pathologically staged by the AJCC guidelines (ajcc.org).

The surgical approach is dictated by a combination of tumor location and surgeon preference. The approach will usually differ based on histology of adenocarcinoma versus squamous cell carcinoma. Most adenocarcinoma are located distally at the gastroesophageal junction. The main approaches for distal esophageal are transhiatal esophagectomy or an Ivor-Lewis esophagectomy. In contrast, most squamous cell carcinomas are located in the mid-thoracic esophagus and require a 3-hole / McKeown esophagectomy to ensure mobilization off of the trachea followed by resection and reconstruction. Allowing this variability is necessary for appropriate standard operative management. Additionally, the approach should have no bearing on the metabolic profiles because the operation will not change the metabolites of pre-nCRT specimens prior to resection.

Jejunostomy tubes are placed in all patients at the NCI. Patients are routinely administered nutrition via the jejunostomy tube until resumption of oral diet which typically takes 10-14 days. For patients with post-operative complications or inability to maintain nutritional goals, jejunostomy feeds are continued after discharge for a variable length of time. For patients with esophageal cancer, this variability cannot be eliminated. Fortunately, the primary endpoints are based on the pre-nCRT biopsies, therefore, post-operative nutritional supplementation will not affect those results. Post-operative specimens will be obtained for secondary endpoints, therefore, whether the patients require prolonged enteral feedings will be noted in analysis. Additionally, post-operative metabolic profiling will focus on time points distant to the operation because full recovery from this operation usually takes six months.

3.4.4 Post-operative Care

Routine standard of care post-esophagectomy practice.

Patient Monitoring:

Initial Monitoring in ICU and transfer to surgical ward as appropriate.

3.5 LONG-TERM FOLLOW UP

Patients will be followed for the secondary endpoints of disease-free survival and overall survival. For patients followed at the NCI, routine clinic appointments and CT scans will be performed at 3, 6, 9, 12, 18, and 24 months then yearly for at least 5 years.

- Patients may continue yearly surveillance and remain on study indefinitely.
- 18F-FDG-PET and CT may be combined or substituted for one another during surveillance.
- Central review of scans in which progressive disease is documented.

Occasionally, surveillance scans for patients undergoing esophagectomy at the NCI are performed at other institutions. The scans and reports will be sent to the NCI for documentation. These scans will be send by mail or electronically to:

Cara M. Kenney, RN, OCD, CCR, NCI
10 Center Drive, CRC Room 4-3752
Bethesda, MD 20892
Phone: 240-760-6233
kenneycara@mail.nih.gov

The data that will be recorded are the disease specific results of CT scans to determine disease recurrence. Overall survival may be recorded by clinic visits or phone calls.

3.6 STUDY CALENDAR

	Screening	**Baseline**	**Neoadjuvant Therapy**	**Pre-Op Evaluation**	**Esophagectomy**	**Post-Op Surveillance**						
						Month[9]						Yearly[7]
						3	6	9	12	18	24	
Histologic Path confirmation	X											
Concomitant Meds		X										
Performance Status[1]	X	X		X								
Nutritional Assessment		X				X	X	X	X	X	X	X
H&P[1]	X	X		X								
EGD[1]	X[6]	X										
Tumor Biopsy[1]	X[6]	X			X							
Normal esophageal biopsy[1]	X[6]	X			X							
CT / PET[1,2]	X	X		X		X	X	X	X	X	X	X
EUS[3]		+ / -										
CBC with platelets[1]	X	X		X		X	X	X	X	X	X	X
Biochemical profile[1, 8]	X	X		X		X	X	X	X	X	X	X
Coagulation labs, CRP[1]	X	X		X								
Pregnancy test[1]	X	X		X								

	Screening	**Baseline**	**Neoadjuvant Therapy**	**Pre-Op Evaluation**	**Esophagectomy**	**Post-Op Surveillance**						
						Month[9]						Yearly[7]
						3	6	9	12	18	24	
PFT[3]		X		X								
EKG[3]				X								
Cardiac w/u if indicated				X								
Research blood (30mL)		X		X[4]	X[10]	X	X	X	X	X	X	X
Research urine (100mL)		X		X[4]	X[10]	X	X	X	X	X	X	X
Chemo/Radiotherapy			X									
Advance Directive [5]		X										

[1] Baseline evaluations will not need to be repeated if they were done at screening within the appropriate timeframe

[2] CT and PET may be combined or substituted for one another.

[3] PFT, EKG, EUS if medically indicated.

[4] within 4 weeks prior to surgery (after neoadjuvant therapy)

[5] Filling out of the Advance Directive will be offered, but obtaining of it is not required. For details see Section **10.3**

[6] If patient does not have available pathology samples from previous biopsies, EGD with biopsy will be performed on Screening

[7] All study subjects will be invited yearly for follow up visit. If patents are not able to come, they will be asked by phone for disease and performance status. Outside tests results will be acceptable.

[8] Biochemical profile: (Sodium (Na), Potassium (K), Chloride (Cl), total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), albumin and pre-albumin

[9] Monthly visits are approximate

[10] On Day 7 after surgery samples for research will be collected

3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

3.7.1 Criteria for removal from protocol therapy:

- Participant requests to be withdrawn from active therapy
- Investigator discretion
- Positive pregnancy test

3.7.2 Off-Study Criteria

- No longer a surgical candidate after nCRT secondary to metastatic disease or deterioration of performance status.
- Participant requests to be withdrawn from study
- Patient lost to follow up
- Investigator discretion
- Death
- PI decision to close the study
- Loss of capacity to give informed consent

3.7.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is off protocol therapy and when a subject is taken off-study. A Participant Status Updates Form from the web site **(**http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

All concomitant medications during neoadjuvant therapy will be recorded. Other medications that are administered routinely with nCRT therapy will not be recorded. All medication will be listed in Labmatrix.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH

Tissue may be used to obtain RNA, DNA, and protein to assay for BH3 profiling, changes in gene expression, metabolism, and protein modification. The specimens of normal esophagus, esophageal tumors, blood, and urine will be analyzed for the metabolic profiles to determine whether specific signatures are associated with the primary outcome of tumor pathological response as defined by the Mandard Score.

5.1.1 Tissue Collection

Specimens of esophageal tumor with matched normal esophagus will be obtained before and after neoadjuvant therapy by endoscopic biopsy or surgical resection. Biopsies of both normal esophagus and tumor will aim for 50 mg – 200 mg of tissue. The tissue will be split for a fresh sample and a frozen sample. The fresh tissue will be sent to the Ripley laboratory for BH3 profiling. Expertise for BH3 profiling analysis will be provided by the Letai laboratory. The other part of the tissue will be placed in Nunc tubes and snap frozen in liquid nitrogen in the operating room. Specimens will be sent to Clinical Pharmacology Program under the direction of Dr. W. Douglas Figg, section **5.2.1**

5.1.2 Blood and Urine Collection

Peripheral blood (30ml) and urine (100ml) samples will be obtained during clinic visits prior to nCRT, within 4 weeks prior to surgery (after nCRT), at day 7 after surgery and at 3, 6, 9, 12, 18, and 24 months then yearly for at least 5 years. Specimens will be sent to Clinical Pharmacology Program under the direction of Dr. W. Douglas Figg, section **5.2.1**

Test/assay	**Volume (approx)**	**Collection point** **(See Study Calendar 3.6)**	**Location of specimen analysis**
Metabolomics analysis/profiling	2 tumor samples, 2 normal esophagus sample (50-100 mg of tissue)	Before and after neoadjuvant therapy	Metabolon[1]
Pathological response	Esophageal surgical specimen	Surgery	Dr. Quezado[2] Laboratory of Pathology
P53 mutational analysis (sequenced by single-nucleotide polymorphism (SNP)) RNA sample	1 tumor sample, 1 normal esophagus sample (50-100 mg of tissue	Before neoadjuvant therapy	RNA generated in Dr. Ripley laboratory and send for analysis to Genewiz LLC[3]
RNA analysis (sequenced by single-nucleotide	1 tumor sample, 1 normal esophagus	Before neoadjuvant therapy	RNA generated in Dr. Ripley laboratory and send for analysis to RNAseq to NCI, Frederick[4]

Test/assay	**Volume (approx)**	**Collection point (See Study Calendar 3.6)**	**Location of specimen analysis**
polymorphism (SNP))	sample (50-100 mg of tissue		
Metabolomics analysis/profiling	Blood 30 ml	Before and after neoadjuvant therapy	Metabolon[1]
Metabolomics analysis/profiling	Urine 100 ml	Before and after neoadjuvant therapy	Metabolon[1]
BH3 protein profiling	2 tumor samples, 2 normal esophagus sample (50-100 mg of tissue	Before and after neoadjuvant therapy	Dr. Ripley laboratory
whole genome/whole exome sequencing	Leftover from tumor Biopsy and/or blood	Before neoadjuvant therapy (optional)	DNA samples generated in Dr. Ripley laboratory. Facility for genome sequence will be defined after all samples are collected

[1]Metabolon: (Contact: John Luster, NIH/NIEHS Science Development Director, m. 919.627.2465, f. 919.287.2677; (JLuster@metabolon.com) Metabolon, Inc., PO Box 110407, Research Triangle Park, NC 27709, 617 Davis Dr., Suite 400, Durham, NC 27713) (ZIA BC 011630: Metabolomic Profiling of Esophageal Cancers).

[2] The pCR and Mandard Score[16] will be assessed by the NCI pathologist (Dr. Martha Quezado). Given that the metabolic signatures will be developed after treatment response assessment; the pathological analysis is effectively blinded.

[3] Genewiz Project Management, 115 Corporate Boulevard, South Plainfield, NJ 07080 (877-436-3949)

[4] Bao Tran, Director, Sequencing Facility; (https://ostr.cancer.gov/resources/fnl-cores/sequencing-facility); Leidos Biomedical Research, Inc.; Frederick National Laboratory for Cancer Research (FNLCR); 8560 Progress Drive, Room D3047, Frederick, MD 21701 (301-360-3460); tranb2@mail.nih.gov). Quality assurance will be performed on RNA samples prior to shipping to Frederick by Val Bliskovsky (301-435-7249; bliskovv@mail.nih.gov; 37 Convent Drive, Rm 2135, Bethesda MD 20892).

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without IRB notification and an executed MTA.

All samples will be sent to Blood Processing Core (BPC) for processing and storage until they are distributed to Dr. Ripley's lab or to the designated facility as described in the protocol.

5.2.1 Samples Managed by Dr. Figg's Blood Processing Core (BPC)

5.2.1.1 BPC contact information

Please e-mail Julie Barnes at Julie.barnes@nih.gov and Paula Carter pcartera@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact Julie Barnes by e-mail or at 240-760-6044.

5.2.1.2 Sample Data Collection

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in the LABrador (aka LabSamples) utilized by the BPC. This is a secure program, with access to LABrador limited to defined Figg lab personnel, who are issued individual user accounts. Installation of LABrador is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen. All Figg lab personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.

LABrador creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without LABrador access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.2.1.3 Sample Storage

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in LABrador. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

5.2.2 Procedures for storage of tissue specimens in the Laboratory of Pathology

Tissues designated for clinical diagnostics are transported to the Laboratory of Pathology (LP) where they are examined grossly and relevant portions are fixed, embedded in paraffin and sectioned and stained for diagnostic interpretation. Unutilized excess tissue that is not embedded in paraffin is stored in formalin for up to three months, in accordance with College of American Pathologists/Joint Commission on Accreditation of Healthcare Organizations (CAP/JCAHO) guidelines, and then discarded. Following completion of the diagnostic workup, the slides and tissue blocks are stored indefinitely in the LP's clinical archives. All specimens are catalogued and retrieved utilizing the clinical laboratory information systems, in accordance with CAP/JCAHO regulations. The use of any stored specimens for research purposes is only allowed when the appropriate IRB approval has been obtained. In some cases, this approval has been obtained via the original protocol on which the patient was enrolled.

5.2.3 Procedures for storage of specimens in the Laboratory of Dr. Taylor Ripley

Samples will be stored in the locked refrigerator with limited access to the lab personnel with completed trainings. Samples will be tracked in the excel file (Patient Samples – Metabolomics Trial 17-C-0135) and stored on the PI's server (ripleyr) only. We will maintain a separate Excel file (Patient Samples – Metabolomics Trial 17-C-0135 Deidentified) on the laboratory server (Thoracic and GI Oncology Branch) with patients' information coded by number that requires the PI's data to de-identify.

5.2.4 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described in the section above. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. The PI will report any loss or destruction of samples to the NCI IRB as soon as he is made aware of such loss.

If the patient withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

After specimens from all patients have been obtained, the tissue, blood, and urine will be sent as de-identified specimens to the company, Metabolon. We anticipate that the accrual goal will be met in four years. The specimens will be analyzed and the data interpreted by Metabolon to determine whether the changes in metabolites provide a signature that is predictive of response to therapy.

Once the accrual goal has been met, the protocol will remain open to continue to follow patients for the secondary endpoints of disease-free survival and overall survival. Additional samples that are not sent to Metabolon, to Genewiz, or for RNAseq will remain in storage. We will transfer

them to a specimen storage study to keep the specimens indefinitely. At the conclusion of this protocol, if additional studies are to be performed on any samples obtained during the conduct of this trial, a Request to Conduct Research for Stored Human Samples Specimens, or Data Collected in a Terminated NCI-IRB Protocol will be submitted.

5.3 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

5.3.1 Description of the scope of genetic/genomic analysis

The p53 mutational status will be determined as a correlative study. The p53 mutational status will be sequenced by single-nucleotide polymorphism (SNP) discovery/mutational analysis by Genewiz® on tumor samples and possibly normal esophageal samples. p53-mutant or p53 wild-type will be recorded. Furthermore, the specific mutations such as the DNA binding mutation, R175H, and the protein folding mutation, R248W, will be determined. This analysis will be exploratory and help determine whether to pursue different therapeutic strategies based on the specific p53 mutations.

5.3.2 Future whole genome/whole exome studies

Further studies may be conducted in the future in order to genotype samples to predict response and/or toxicities to other investigational agents. All patients may undergo whole genome sequencing.

No specific sample collection will be made for potential whole genome/exome studies. Leftover samples from other procedures including biopsies and blood samples may be used for this purpose.

5.3.3 Privacy and confidentiality of medical information/biological specimens

Confidentiality will be maintained as described in section **5.2**. At the time of analysis, samples are transferred to the company, Metabolon. No patient identifiers (e.g., medical record number, patient name or initials) will be included with the sample. Metabolon will have unique sample ID's that can be linked only by the PI or AI's of the study.

No personally identifiable information will be released to third parties and samples and data will only be shared with other researchers with the permission of the IRB and under the proper Material Transfer Agreements.

5.3.3.1 Certificate of Confidentiality

As part of study efforts to provide confidentiality of subject information, this study has obtained a Certificate of Confidentiality which helps to protect personally identifiable research information. The Certificate of Confidentiality allows investigators on this trial to refuse to disclose identifying information related to the research participants, should such disclosure have adverse consequences for subjects or damage their financial standing, employability, insurability or reputation.

5.3.4 Management of Results

The analyses performed in various NCI laboratories under this protocol are for research purposes only; they are not nearly as sensitive as the tests that are performed in a laboratory that is certified to perform genetic testing for clinical purposes. Changes observed unrelated to our research may or may not be valid. Therefore, we do not plan to inform participants of the results of testing on the tissue and blood that is performed in our research lab. However, in the unlikely event that

clinically relevant incidental findings are discovered, subjects will be contacted if a clinically actionable gene variant is discovered.

Clinically actionable findings for this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists)

Subjects that remain on the study will be contacted at this time with a request to provide a blood sample to be sent to a CLIA certified laboratory. If the research findings are verified in the CLIA certified lab, the subject will be referred to an NCI CCR Genetics Branch certified genetic health care provider for the disclosure of the results.

This is the only time during the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

The costs of CLIA testing will be paid for by the Center for Cancer Research, the Branch, or the Principal Investigator. If the health history, family history, or tumor diagnosis from the Laboratory of Pathology at the NIH Clinical Center suggests that the participant might benefit from genetic testing, we will discuss this with him/her.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

As this is NOT a research treatment study any data collection will include demographic information, patient history; including risk factors, details of the surgical intervention, and listing of treatments given. The results of clinical procedures/tests will be kept in the patient's permanent medical record. At the request of the patient, the results of the test/procedures can be communicated to the patient's referring physician. Blood and tissue specimens collected during this research project may be banked and used in the future to investigate new scientific questions related to this study. Any new use of research specimens will be review and approved by the IRB or OHSRP. Adverse events data will not be collected for the standard of care procedures.

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

Labmatrix will be used for sample data collection. Data in this system will be entered by the NCI staff. Clinical data will be captured in C3D.

End of study procedures: Data will be stored according to HHS, FDA and NIH Intramural Records Retention Schedule regulations as applicable.

Loss or destruction of data: Should we become aware that a major breech in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

I will share human data generated that is de-identified in an NIH-funded or approved public repository at the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.3 RESPONSE CRITERIA

The patients will undergo nCRT for resectable disease, therefore, they will be NED after treatment. The patients will be followed for disease-free survival which will be defined as the appearance of any new lesion that is likely metastatic or locally-recurrent esophageal cancer and overall survival.

6.3.1 Definitions

Evaluable for toxicity: No toxicity other than standard measures is relevant because this trial is a biomarker trial.

Evaluable for objective response: There is no evaluation for objective response because these patients have resectable disease.

6.3.2 Methods for Evaluation of Recurrent Disease

Conventional CT: Recurrence of disease will be evaluated by routine surveillance imaging or symptoms warranting clinical evaluation. MRI is also acceptable in certain situations although rarely necessary for this trial.

18F-FDG-PET: Patients will have PET scans prior to and after nCRT therapy. New lesions on post-operative CT will usually be evaluated by FDG-PET imaging. Post-operatively, patients will only receive PET scans if CT scans have suspicious lesions or patient has new symptoms suggestive of metastatic or recurrent disease.

6.3.3 Duration of Response

Duration of overall response and Duration of stable disease: Not relevant to this trial.

6.3.4 Disease-Free Survival (Recurrence-Free Survival)

Disease-free survival will be defined from the time of esophagectomy until development of metastatic disease or death, whichever comes first. Usually, post-operative CT surveillance will detect new metastatic disease, however, symptoms warranting evaluation will be evaluated as clinically appropriate.

6.4 TOXICITY CRITERIA

No experimental treatments or procedures are performed as a part of this protocol and therefore no adverse events are expected.

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.2 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

7.1.3 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.4 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and

  (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NCI-IRB AND NCI CLINICAL DIRECTOR REPORTING

7.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received by the NCI-IRB within 7 days of PI awareness via iRIS.

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

This is not a research study and no data are collected on the subjects enrolled on this protocol other than basic demographic information at the time of enrollment. Therefore, no information about adverse events (other than deaths on study) will be available to the PI at the time of continuing review. Each subject treated on this protocol will receive individualized treatment with conventional treatments, and as a result a systematic analysis of adverse event or response data would not be possible and would not impact on the treatment of future subjects. The following will be provided at the time of continuing review:

- A summary of deaths on study.
- A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
- A summary of any instances of non-compliance

7.3 DATA AND SAFETY MONITORING PLAN

7.3.1 Principal Investigator/Research Team

The clinical research team will meet monthly when patients are being actively treated on the trial to discuss each patient.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS.

The principal investigator will review response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 STATISTICAL CONSIDERATIONS

The primary objective is to determine whether a metabolomic signature in tumor, blood, or urine or whether BH3 profiling of pre-neoadjuvant tumor biopsy correlates with the outcome of pCR after nCRT for patients with EAC or ESCC. The measurement of theprimary endpoint is whether or not viable tumor is present after surgical resection – pCR. Similarly, a secondary objective is to identify whether metabolomic signatures in tumor, blood, or urine or BH3 profiling in tumor of EAC and ESCC patients correlate with major responses (Mandard score of 1 and 2) versus minimal response (Mandard score 3-5). This analysis is also based on pathological findings of the surgical resection. The patients with major responses include <10% viable tumor and pCR versus patients with any grade over 10% viable tumor. Another secondary objective is descriptive to evaluate metabolomic profiles of these patients as an exploratory analysis to determine whether certain pathways are significantly upregulated in esophageal cancer.

Analyses involving the actual metabolomics profiles, as well as the analyses involving metabolic signatures, will be done by individuals with expertise in this area of high throughput data science, using methodology appropriate to that field. The comparison of metabolomic profiles between those with a pCR and the other patients will be done using appropriate bioinformatics procedures. Since this trial is an exploratory biomarker trial, the true number of patients to power this study is

unknown. Sreekumar and colleagues compared 16 prostate normal tissue samples to 12 samples of localized prostate cancer to 14 metastatic prostate samples and successfully identified metabolites associated with progression in prostate cancer (**Figure 3**).[26] The NIH Common Fund Eastern Regional Comprehensive Metabolomics Resource Core (ERCMRC) has a group with the expertise to process and analyze this data once all the specimens have been collected. They will collaborate with this project. We will plan to accrue 10 patients with pCR for both EAC and ESCC in order to have a minimal number of patients with pCRs to compare against the other subjects. For patients with EAC or ESCC, the percentage of patients with a pCR after nCRT is well-documented and significantly different between them. Therefore, patients with EAC and ESCC will be evaluated independently in two cohorts. Patients with EAC are reported to have 17-27% of pCR.[3, 5, 11, 12, 20] Assuming 20% of patients have a pCR with EAC, 66 patients will be accrued in order to have 86% probability of obtaining 10 patients with pCR. Thus, the accrual goal for Cohort 1 for EAC will be set at 66 evaluable patients, and will have an accrual ceiling of 80 patients to allow for up to 14 inevaluable cases. Patients with ESCC are reported to have 40-64% of pCR after neoadjuvant CRT.[13, 15, 45] Assuming 40% of patients have a pCR with ESCC, 32 evaluable patients will be accrued in order to have 88% probability of obtaining 10 patients with pCR. Thus, the accrual goal for cohort 2 will be set at 32 evaluable patients, and will have an accrual ceiling of 40 patients to allow for unevaluable cases. The overall accrual ceiling of the entire study will be 120 patients to allow for to 22 unevaluable patients.

The secondary outcomes will be the association of overall survival (OS), disease-free survival (DFS), pathological stage (ypStage), and p53 mutational status with a metabolic signature. Additionally, the patients will be divided by pathological major response (Mandard 1-2) compared to minor or no response (Mandard 3-5). This analysis is similar to comparison of pCR to non-pCR, however, patients with <10% viable tumor will be included in the favorable group; therefore, this group will be slightly larger than 10 patients with pCR. The OS and DFS will be calculated by Dr. Seth Steinberg using Kaplan-Meier and log-rank tests. The final, pathological stage will be reviewed by the PI prior to any analysis. Given that multiple stages are possible, the additional subgroup analysis of ypStage will be reported as descriptive statistics only without metabolic analysis.

To allow for a small number of unevaluable patients, the accrual ceiling will be set to 120 patients for the entire study.

The accrual ceiling will be 80 patients for EAC. If 1 patient every month enrolls onto this study, accrual is expected to be completed in 6-7 years.

The accrual ceiling will be 40 patients for ESCC. If 1 patient every 3-4 months enrolls onto this study, the accrual is expected to be completed in 10 years.

9 COLLABORATIVE AGREEMENT

Material Transfer Agreement (MTA)

An MTAs with Metabolon and Genewiz LLC will be in place to perform the studies on de-identified samples as indicated in section **5.1**

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

The research subject selection is expected to reflect the epidemiology of EAC in the United States. The disease affects men about 6-8x more often than women. The disease occurs most often in patients with the risk factors of history of reflux, obesity, and cigarette smoking. No exclusion criteria exist for gender, ethnicity, or race as long as the patient is deemed fit for neoadjuvant chemoradiotherapy and surgery.

The recruitment plan is similar to building a physician practice. Most patients will be referred from institutions in the local area from surgeons and gastroenterologists whom already refer patients to our group of thoracic surgeons. This protocol will be submitted to Trials magazine for publication to be searchable on PubMed.

10.2 PARTICIPATION OF CHILDREN

Children are excluded because esophageal adenocarcinoma in children is exceedingly rare and we do not expect any children to be diagnosed with this disease. Boys and colleagues reviewed 772 patients with esophageal cancer and the youngest patient was 30 years old.[46]

10.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisional impaired. Because there is no prospect of direct benefit from research participation section **10.4**, subjects will be removed from study participation if they become incapacitated or cognitively impaired during the course of the study.

10.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

Benefits, risks/discomforts will be carefully explained to the patient based on the treatment decided upon by the CCR investigator.

10.4.1 Risks/Benefits Analysis

There is no direct benefit from participating in the study. Risks will be specific to medical care recommended to the patient and will be documented in the consent form.

The risks of each treatment/procedure/test, as well as the implications of the treatment/procedure/test results will be discussed thoroughly with the patient prior to the intervention. Additional consents will be obtained for any procedures performed as appropriate. The biopsies performed on this trial are performed as standard of care regardless of trial participation.

10.5 CONSENT PROCESS AND DOCUMENTATION

All patients are thoroughly screened prior to initial consultation at the NIH. This usually involves a telephone conversation between the patient and a physician or nurse associate investigator. During the initial consultation, the patient, along with family members, is presented a forthright and detailed overview of the study plan. The Informed Consent document is given to the patient and they are asked to review it, make notes and follow-up with a phone call to the physician or

nurse investigator to have any additional questions answered prior to considering treatment on protocol.

When the patient is admitted for treatment, an associate physician investigator responsible for the care of the patient presents the previously described information in detail. The research nurse or Principal Investigator, or designee is responsible for obtaining consent from the patient upon admission. The patient is reassured that participation on the trial is entirely voluntary and that they can withdraw or decide against treatment at any time without adverse consequences. In fact, the investigators assure the patient that if alternate therapies are preferred that we will do all that we can to facilitate obtaining consultation and treatment from the appropriate medical center. The signed consent will be verified by the physician responsible for the care of the patient. The patient is asked to participate in completing the self-administered questionnaires measuring health related quality of life during this study. They are assured that their eligibility to participate in the perfusion portion of this study is not dependent upon their willingness to complete the quality of life questionnaires.

10.5.1 Re-consent via Telephone

The informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone. A fully executed copy will be returned via mail for the subject's records.

The informed consent process will be documented on a progress note by the consenting investigator.

10.5.2 Consent of non-English Speaking Subjects

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OHSRP SOP 12, 45 CFR 46.117 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation (using either the long translated form or the short form). Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process for non-English speaking subjects and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

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12 APPENDICES

12.1 APPENDIX A -PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.