PROSPECTIVE DETECTION OF CHEMORADIATION RESISTANCE IN PATIENTS WITH LOCALLY ADVANCED ESOPHAGEAL ADENOCARCINOMA

A thesis submitted to the University of Arizona College of Medicine – Phoenix
in partial fulfillment of the requirements for the Degree of Doctor of Medicine

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Class of 2017

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Acknowledgements

Castle Biosciences Inc. provided funding for this project. Thank you to the various professionals employed by Castle Biosciences Inc. who made this project possible: John F Stone PhD, Jeff Wilkinson PhD, Natalie Lassen PhD, Clare Johnson RN, Kristen M. Oelschlager RN, Derek Maetzold BS, Robert W. Cook PhD and Weiwei Shan PhD.

Special thanks to the following clinical collaborators: Paolo Cotzia MD and Adam C. Berger MD of Thomas Jefferson University, Daniel Rosen MD of Baylor College of Medicine and Sunil S. Badve MD, Romil Saxena MD, Juan Palazzo MD and Kenneth Kessler MD of Indiana University.

All supplies were provided through the Molecular Medicine Laboratory at St. Joseph's Hospital and Medical Center.

Abstract

Background: Approximately 25% of patients with locoregional esophageal adenocarcinoma (EC) are resistant (marked by minimal tumor regression; TRG 3) to preoperative chemoradiation, including 5FU-based and CROSS regimens. Previously, an immunohistochemistry (IHC) test that accurately identifies patients as responders (TRG 0-2) or non-responders (TRG 3) to neoadjuvant CTRT was developed and validated. The current study was designed to identify gene expression profile (GEP) signatures able to predict response to preoperative treatment. Methods: Formalin-fixed, paraffin-embedded (FFPE) tumor tissue from 24 diagnostic biopsies (14 responders, 10 non-responders) was collected. RNA was isolated, and RT-PCR performed to assess the expression of 96 candidate genes chosen from in silicoanalysis. Genetic signatures incorporating genes with significant expression differences in pathologically determined responders versus non-responders were identified, and linear and non-linear predictive modeling methods were used to assess the accuracy of the signatures for predicting treatment response. Cross validation was performed to attain corrected accuracy values. Results: Ten-, 18-, and 24-gene signatures were identified with significantly different gene expression levels in responders compared to non-responders ($p < 0.05$). Functional groups represented by the signatures included DNA damage repair, extracellular matrix remodeling, and 5FU metabolism. Partial Least Squares (PLS) prediction of treatment response was compared to pathologic TRG determined by blinded pathologic reading, and resulted in an area under the curve (AUC) of 0.99 and overall accuracy of 100% for the 24-gene signature. Corrected AUC of 0.99 and accuracy of 95% resulted from five-fold cross validation with 20 iterations. Heatmap analysis of the 24-gene signature separated the EC cases into two distinct clusters, the first with 93% responders and the second with 90% non-responders. Conclusions: The current study identifies novel gene signatures able to accurately predict EC patient response to preoperative treatment. The GEP may allow non-responders to avoid unnecessary toxicities associated with chemoradiation therapy.

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Introduction, Significance and Rationale

Esophageal cancer affects the global population, being the 8th most common cancer in the world. Incidence has been increasing, as with mortality, in the last 20 years. Prognosis is poor, the 2-year survival being only 43%, when using the standard treatment plan of neoadjuvant chemotherapy and resection surgery.[1] As clinically observed, there are responders and non-responders of esophageal carcinoma to neoadjuvant chemotherapy. This non-responder group could benefit from a more aggressive treatment option if identified.[1]

Patients with locoregional esophageal adenocarcinoma have been identified as responders and non-responders to neoadjuvant chemoradiotherapy with immunohistochemistry (IHC).[2] Rosen et al. demonstrated that IHC can be used as a predictive test for patients, with 82-88% positive predictive value and 83% negative predictive value.

A previous study demonstrated that a gene expression signature can predict response to neoadjuvant chemotherapy (n=20).[3] This initial evidence would suggest that certain genes are associated with poor prognosis in this type of esophageal cancer. By applying previous knowledge of ESCC, this research will look at biopsies of esophageal adenocarcinoma to determine a gene signature specific to response to neoadjuvant chemotherapy.

Rao et al. found a “poor outcome” group and “good outcome” group of cancer patients with adenocarcinoma of the esophagus and esophagogastric junction treated with preoperative chemotherapy based on gene expression profiles. Looking at 165 genes, it was found that 47 genes were differently expressed between groups (n=35).[1] A larger sample size using a 96-gene panel developed by Castle Biosciences Inc. was tested to determine if it could identify groups more distinctly with fewer target genes.

Based on the previous immunohistochemical and gene expression findings, we hypothesized that there should be a significant genetic expression difference amongst esophageal adenocarcinoma biopsies that correlate with the response to neoadjuvant chemotherapy.

Research Materials and Methods

The methods described below will help answer the question: "Can a genetic expression signature be developed to predict the response to neoadjuvant chemotherapy in esophageal adenocarcinoma?" It is expected that using a microarray 96-gene panel will generate data that will discriminate esophageal adenocarcinoma into 2 groups based on known outcomes: responders and non-responders. It is also possible that genetic expression can yield no statistically significant differentiation or that it will create more than 2 groupings. Alternative gene panels and using a larger sample size could be explored, if necessary, in another project.

Tumor Samples

There were 24 samples with known outcomes in this study, 14 responder and 10 non-responder formalin-fixed, paraffin-embedded (FFPE) sections of esophageal adenocarcinoma. The tumor samples had been collected and appropriate informed consent was obtained for all non-decedent samples. This number was determined to be sufficient to yield statistically significant results to see major groupings of gene expression by the Castle Biosciences, Inc. biostatistics group. The biopsy specimens were diagnostic of patients who were subject to neoadjuvant chemotherapy and tumor resection. The biopsies received for experimentation were assigned coded ID and labeled non-responder or responder so that PHI is protected. The number of 5µm FFPE sections per patient was 10 wherever possible. The biopsies were collected from various institutions including Thomas Jefferson University, Baylor College of Medicine and Indiana University Medical Center (all IRB approved).

Preparation of RNA Samples

Tumor samples 10 FFPE sections were obtained from each sample tissue block. One section was stained with hematoxylin and eosin (H&E) to localize the areas of tumor within the sections. Tumor tissue was dissected away from normal-appearing tissue. Total RNA was isolated using Molecular Medicine Laboratory established protocol.

Real-Time Polymerase Chain Reaction Analysis

RNA samples were reverse transcribed to cDNA and amplified using established protocols.[4,5]

Gene Expression Profiling

A 96-gene panel was analyzed by real-time quantitative PCR (rqPCR), utilizing the Life Technologies 7900HT rqPCR instrument with 384-well TaqMan Low Density Array platform according to the protocol developed by Castle Biosciences, Inc.

Biostatistical Analysis

Castle Biosciences Inc. conducted the principle component analyses. Genetic signatures incorporating genes with significant expression differences in pathologically determined responders versus non-responders were identified, and linear and non-linear predictive modeling methods including partial least squares (PLS) were used to assess the accuracy of the signatures for predicting treatment response. Cross validation was performed to attain corrected accuracy values.

Figure 1 demonstrates the methods of this project in visual form.

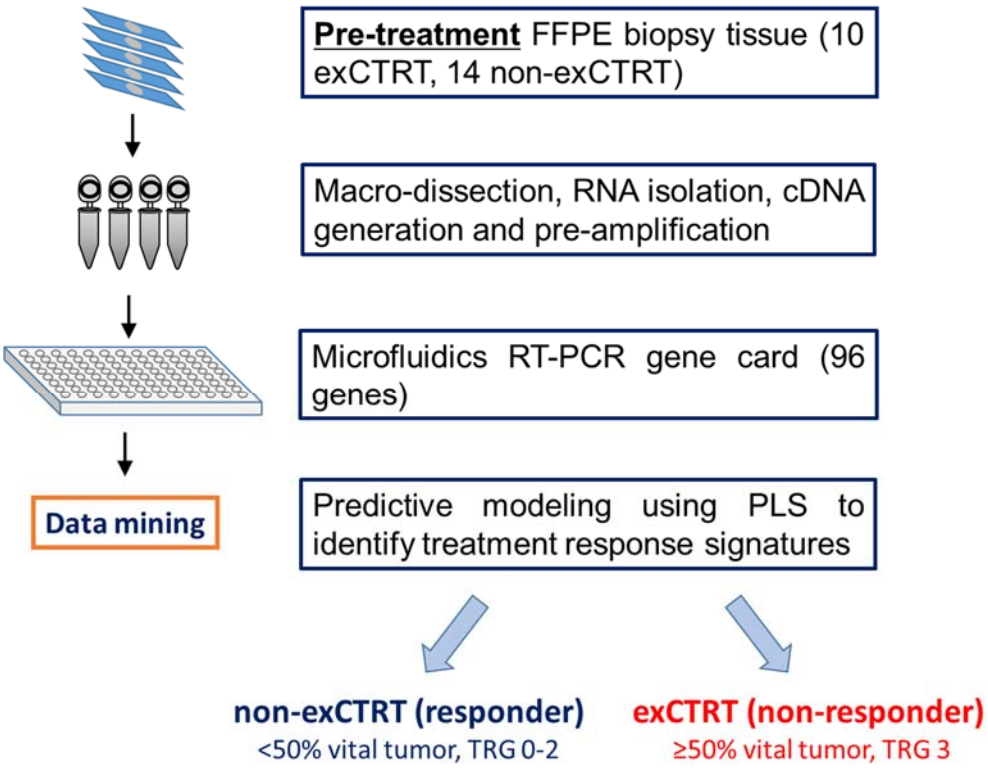

Figure 1. Methods

Results

Figure 2 demonstrates the patient demographics associated with the 24 samples collected for this project. The majority of samples were from male patients with T3N1Mx staging of esophageal adenocarcinoma. 18 out of the 24 patients had confirmed neoadjuvant 5-FU chemotherapy.

There were 96 genes tested for the gene expression profile. The 96 genes tested are found in various pathways related to cancer growth and chemotherapy metabolism. Functional groups represented by the signatures included DNA damage repair, extracellular matrix remodeling, and 5-FU metabolism (Figure 3). A number of genes were discriminatory between the responder and non-responder groups. Ten-, 18-, and 24-gene signatures were identified with significantly different gene expression levels in responders compared to non-responders ($p < 0.05$).

Heatmap analysis of the 24-gene signature separated the esophageal adenocarcinoma cases into two distinct clusters, the first with 93% responders and the second with 90% non-responders (Figure 4). Each row represents an individual case, each column a proprietary gene. The red represents a gene that is highly expressed, while blue represents down-regulation of a gene.

Partial Least Squares (PLS) prediction of treatment response was compared to pathologic TRG determined by blinded pathologic reading, and resulted in an area under the curve (AUC) of 0.99 and overall accuracy of 100% for the 24-gene signature (Figure 5). Corrected AUC of 0.99 and accuracy of 95% resulted from five-fold cross validation with 20 iterations.

Patient Demographic		#	%
Age	Range	40-75	
	Median	61	
Gender	Male	21	88%
	Female	2	8%
	Unknown	1	4%
Baseline T Stage	Tx	1	4%
	T1	2	8%
	T2	3	13%
	T3	18	75%
Baseline N Stage	Nx	2	8%
	N0	8	33%
	N1	10	42%
	N2	2	8%
	N3	2	8%
Baseline M Stage	Mx	21	88%
	M0	2	8%
	M1	1	4%
Neoadjuvant CT regimen	5-FU	18	75%
	Non 5-FU	1	4%
	Unknown	5	21%
Pathologic Tumor Response	exCTRT (TRG 3)	10	42%
	Non-exCTRT (TRG 0-2)	14	58%

Figure 2. Patient Demographics

GO Term	# genes
Regulation of apoptosis	14
Organ development	19
Regulation of cell migration	10
Regulation of epithelial cell proliferation	9
Reproductive structure development	10
Response to organic cyclic compound	12
Regulation of macromolecule metabolic process	17
Response to endogenous stimulus	14

Figure 3. Gene Ontology Analysis

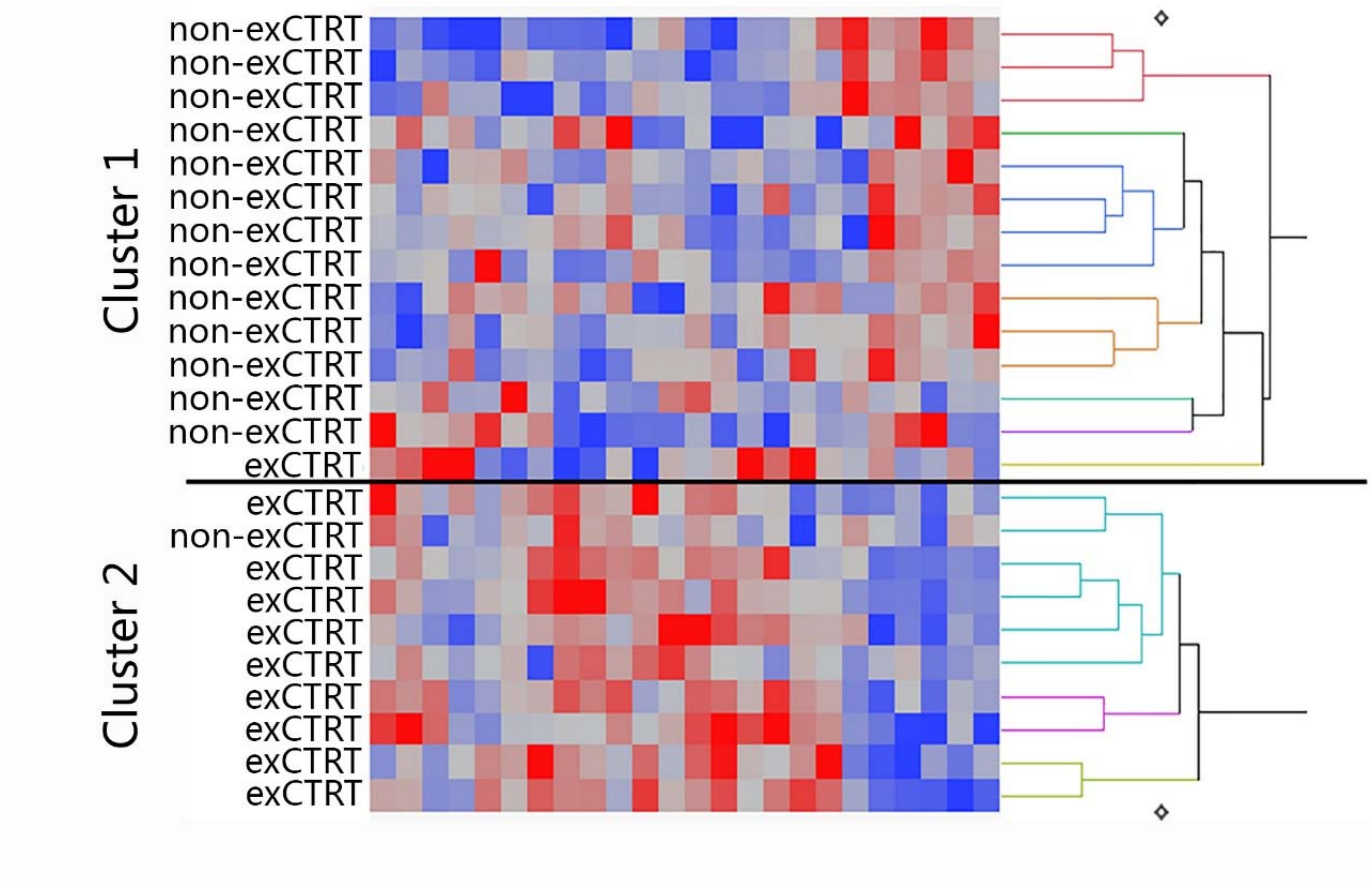

Figure 4. Heatmap analysis, 24 genes, 24 cases

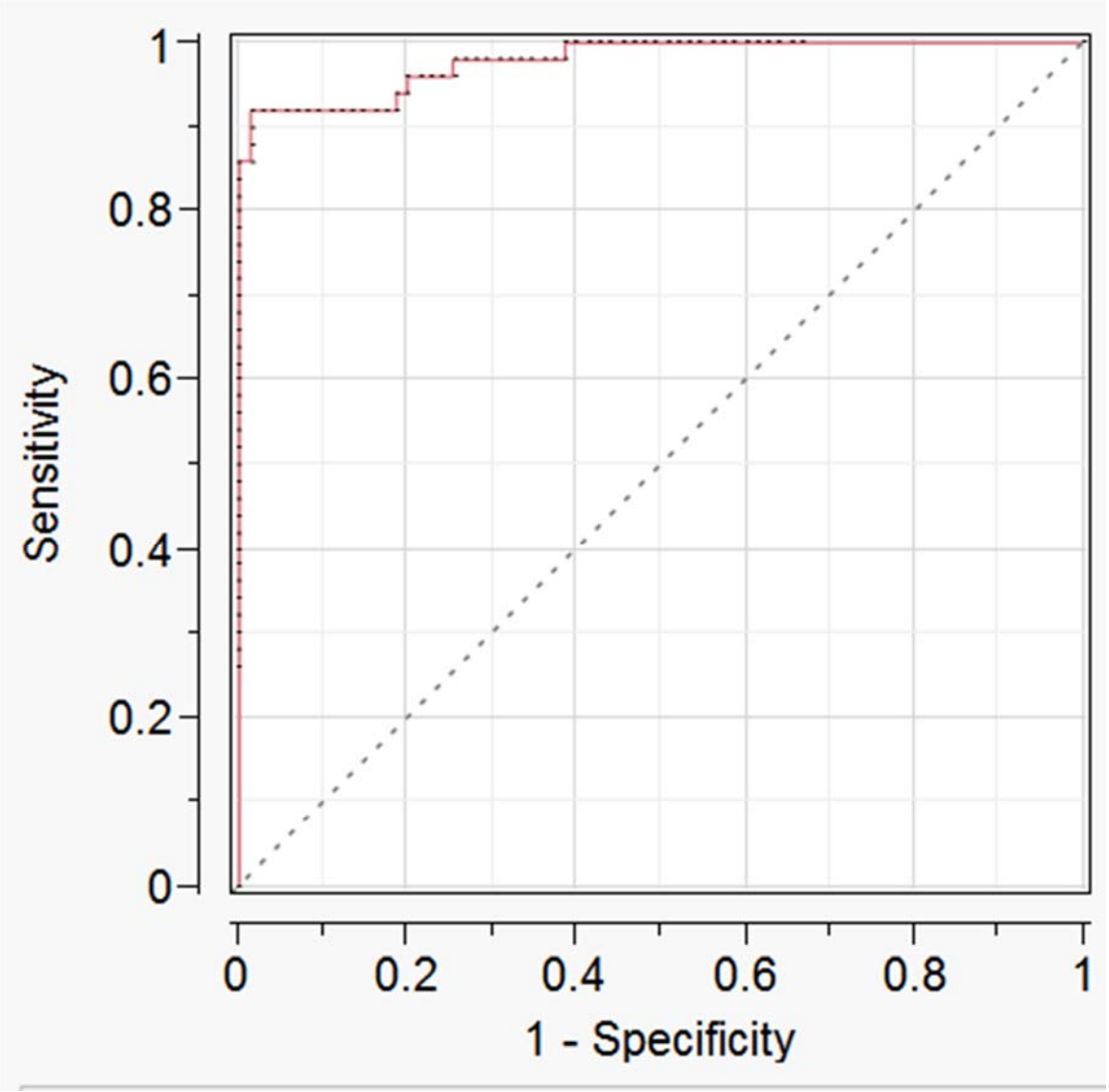

Figure 5. Area Under the Curve (AUC)

Discussion

The standard of care for locoregional esophageal adenocarcinoma (EC) patients is a trimodality approach consisting of pre-operative chemotherapy (CT) and radiotherapy (RT) followed by surgery. Approximately 25-30% of EC patients exhibit extreme resistance to standard CTRT (exCTRT), including 5-FU-based and CROSS regimens. Resistance is marked by minimal tumor regression (Tumor Regression Grade 3).

There is a previously validated immunohistochemistry (IHC) test that accurately identifies patients as responders (TRG 0-2) or non-responders (TRG 3) to neoadjuvant chemoradiation.[2] This current study was designed to identify gene expression profile (GEP) signatures able to predict response to preoperative treatment. If these non-responder (exCTRT) patients could be identified at the time of diagnosis, they could benefit from alternative neoadjuvant regimens or move directly to surgery, avoiding the negative effects of standard treatments. GEPs are currently used clinically, especially in the oncology field.[4,5]

In this experiment the outcome was binary – there is or there is not a genetic expression signature that correlates with responder or non-responder groups of esophageal adenocarcinoma to neoadjuvant chemotherapy.

We hypothesized that there was a significant genetic expression difference amongst esophageal adenocarcinoma biopsies that correlate with the response to neoadjuvant chemotherapy. A genetic expression signature was found that predicts whether a patient's esophageal adenocarcinoma will respond to neoadjuvant chemotherapy. The current study identifies novel gene signatures able to accurately predict EC patient response to preoperative treatment.

Future Directions and Conclusions

There is evidence to suggest that specific expression at the protein level is associated with a response to neoadjuvant chemotherapy.[2] Determining and quantifying the RNA level expression, and subsequently create a GEP, could increase sensitivity and specificity so that more tumor biopsies of adenocarcinomas can be determined responsive or nonresponsive to neoadjuvant chemotherapy.

The development of a gene expression signature to predict the response of neoadjuvant chemotherapy in esophageal carcinoma has benefits for patients and the healthcare industry. A robust predictor of response would obviate the need for all esophageal carcinomas to receive the conventional neoadjuvant chemotherapy, since the clinician can find out if the tumor will respond positively or not to the chemotherapy through the genetic expression signature determined via biopsy. The GEP may allow non-responders to avoid unnecessary toxicities associated with chemoradiation therapy. These results can change clinical practice by creating a new neoadjuvant chemotherapy protocol for esophageal adenocarcinoma (Figure 6). This is a move toward personalized medicine for patients with esophageal adenocarcinoma. Ultimately morbidity can be decreased and health care costs reduced.

Before this state can be reached, large-scale clinical trials will, of course, be required. The 24-gene signature needs to be independently verified by gathering a large number of diagnostic EC tumor biopsies with known outcomes, running the GEP and comparing the test results with the known outcomes. This can create a positive and negative predictive value for the GEP, a needed characteristic of a diagnostic test for clinicians.

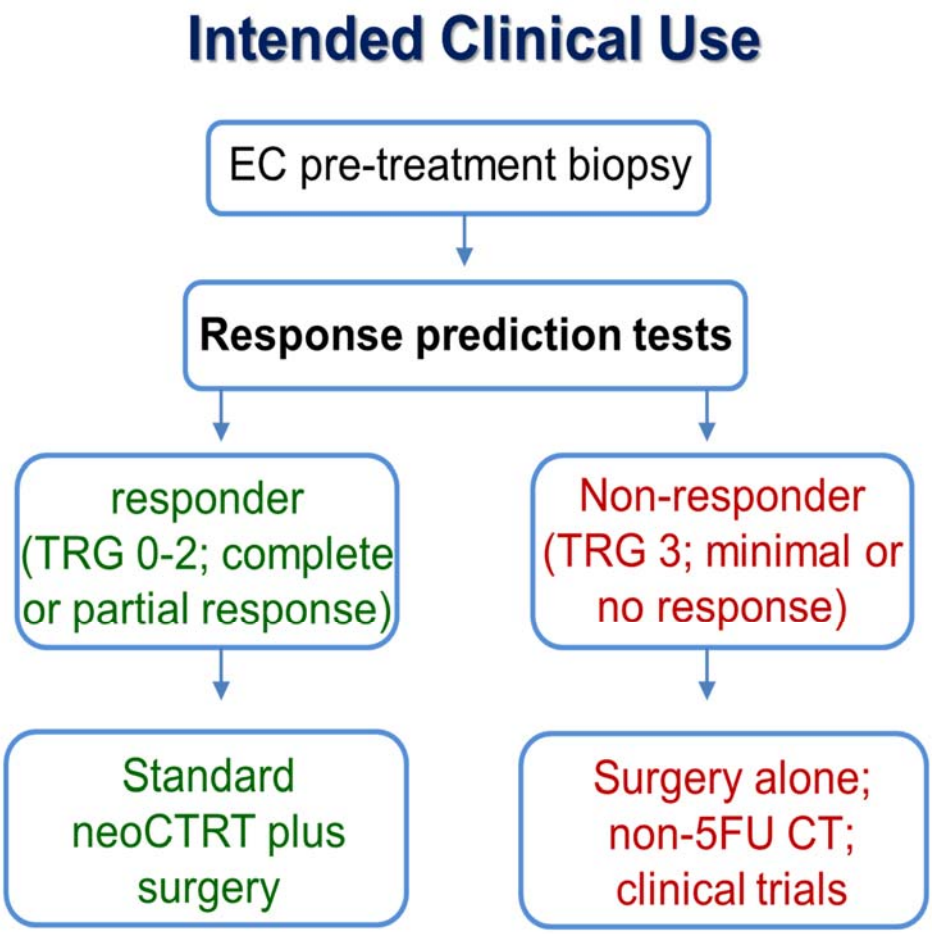

Figure 6. Intended Clinical Use

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