Prospective Detection of Chemoradiation Resistance in Patients with Locally Advanced Esophageal Adenocarcinoma

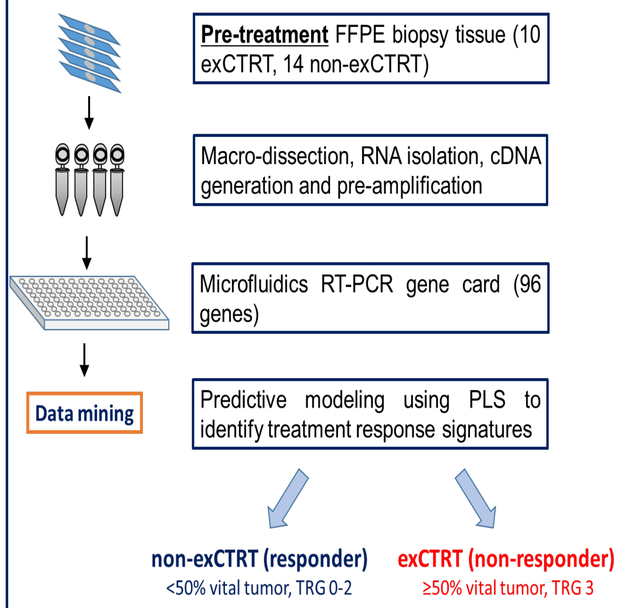
Jennifer M. Veaco MS4[1], Paolo Cotzia MD[2], Adam C. Berger MD[3], Daniel Rosen MD[4], Weiwei Shan PhD[5], Natalie Lassen PhD[6], Clare Johnson RN[6], Kristen M. Oelschlager RN[6], Derek Maetzold BS[6], Sunil S. Badve MD[7], Juan Palazzo MD[8], Robert W. Cook PhD[6], Kenneth Kessler MD[8], Romil Saxena MD[7], Jeff Wilkinson PhD[6, 10], John F Stone PhD[6, 10]

[1]University of Arizona College of Medicine - Phoenix, Phoenix, AZ ; [2]Thomas Jefferson University Hospital, Philadelphia, PA ; [3]Sidney Kimmel Cancer Center at Thomas Jefferson University, Philadelphia, PA; [4]Baylor College of Medicine, Houston, TX; [5]Castle Biosciences Inc., Friendswood, TX; [6]Castle Biosciences Inc., Phoenix, AZ; [7]Indiana University, Indianapolis, IN; [8]Melvin and Bren Simon Cancer Center, Indiana University School of Medicine, Indianapolis, IN; [9]Indiana University School of Medicine, Indianapolis, IN; [10]St. Joseph's Hospital and Medical Center, Phoenix, AZ

Background

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). We have previously validated an immunohistochemistry (IHC) test that accurately identifies patients as responders (TRG 0-2) or non-responders (TRG 3) to neoadjuvant chemoradiation. The current study was designed to identify gene expression profile (GEP) signatures able to predict response to preoperative treatment. If these 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.

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 silico*analysis. 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.

Methods

Patient Demographics		#	%
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 1. Patient Demographics

Results

GO Term	# genes	P value
Regulation of apoptosis	14	2.91E-10
Organ development	19	4.21E-09
Regulation of cell migration	10	2.04E-08
Regulation of epithelial cell proliferation	9	1.15E-07
Reproductive structure development	10	1.32E-07
Response to organic cyclic compound	12	1.38E-07
Regulation of macromolecule metabolic process	17	1.15E-06
Response to endogenous stimulus	14	1.15E-06

Figure 2. Gene Ontology Analysis: 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 5-FU metabolism.

Results

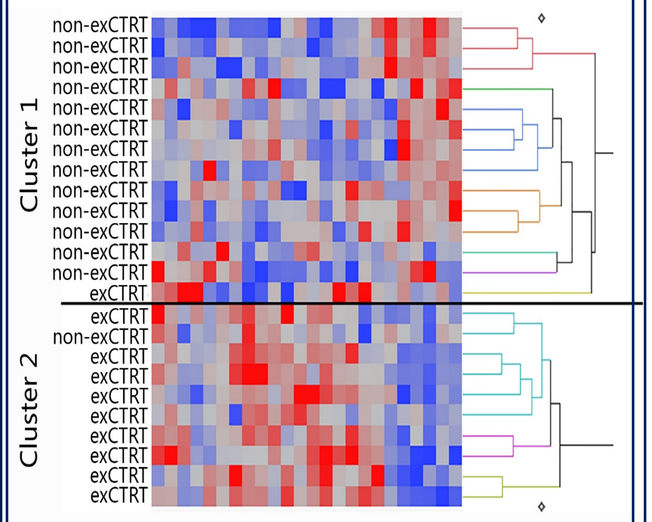


Figure 3. Heatmap analysis, 24 genes, 24 cases: Heatmap analysis of the 24-gene signature separated the EC cases into two distinct clusters, the first with 93% responders and the second with 90% non-responders. Each row represents an individual case, each column a gene.

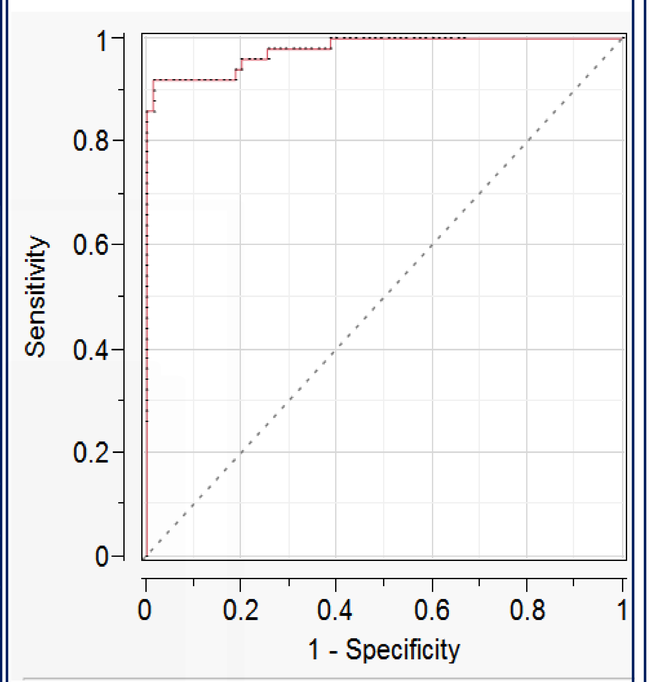
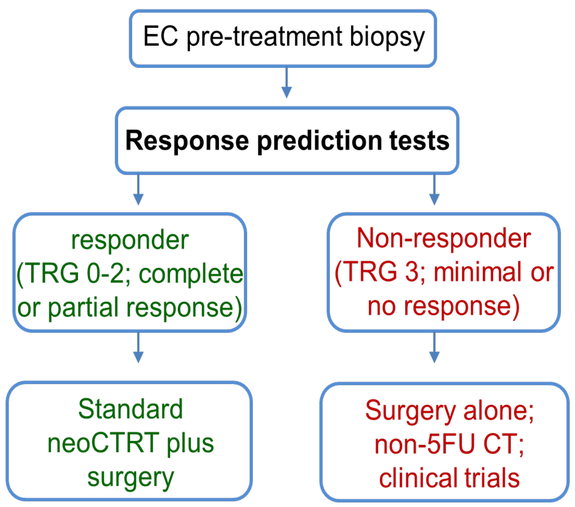


Figure 4. Area Under the Curve (AUC): Partial Least Squares (PLS) prediction of treatment response was compared to pathologic TRG determined by blinded pathologic reading, and resulted in an area under the curve (AUC) of 0.99 and overall accuracy of 100% for the 24-gene signature. Corrected AUC of 0.99 and accuracy of 95% resulted from five-fold cross validation with 20 iterations.

Conclusions

- We hypothesized that there was a significant genetic expression difference amongst EC biopsies that correlate with the response to neoadjuvant chemotherapy.
- A genetic expression signature was found that predicts whether a patient's EC will respond to neoadjuvant chemotherapy.
- 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.
- A robust predictor of response would obviate the need for all ECs 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. Before this state can be reached, large-scale clinical trials will, of course, be required. This is a move toward personalized medicine for patients with esophageal adenocarcinoma. Ultimately morbidity can be decreased and healthcare costs reduced.

Intended Clinical Use



Disclosures & Acknowledgements

WS, NL, CJ, KO, DM, RC, JW and JS are employed by Castle Biosciences. All supplies were provided through the Molecular Medicine Laboratory at St. Joseph's Hospital and Medical Center and funding from Castle Biosciences Inc.