**Assay Description**

**DecisionDx-EC** is a proprietary, multi-analyte assay for esophageal adenocarcinoma which determines a labeling index of the cellular compartment localization of three biomarkers (NF-kB, GLI1, and SHH) from a sample of pre-treatment, diagnostic biopsy tumor tissue. Using a logistic regression algorithm, this intermediate data is then compared to a training set of tumors with known outcomes. The DecisionDx-EC assay reports a likelihood of extreme resistance (exCTRT; CAP Treatment Response Grade 3; no or minimal response) or a likelihood of complete or partial response (non-exCTRT; CAP Treatment Response Grade 0, 1 or 2) to pre-operative chemoradiation therapy (CTRT). CT regimens in the clinical validation studies included combinations of 5-FU and/or taxanes, platinum, folinic acid, CPT-11, or capecitabine; D-FOX, or single therapy 5-FU, taxane, or cetuximab.

**Results**

**DecisionDx-EC = Non-Responder**

**Probability Score:**

<table>
<thead>
<tr>
<th></th>
<th>CAP TRG</th>
<th>% Residual Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-responder (exCTRT)</td>
<td>Grade 3</td>
<td>&gt;50%</td>
</tr>
<tr>
<td>Responder (non-exCTRT)</td>
<td>Grade 0,1 or 2</td>
<td>0% to ≤50%</td>
</tr>
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**Note:** Probability Score does not reflect a p-value, rather it reflects an algorithmic cut-point between the two classifications.

Results should be interpreted using the Clinical Experience information contained in this report, which is derived from clinical studies involving patient populations with specific clinical features as noted in section titled Clinical Experience. These results have not been validated in patients with clinical features different from those described.

Pathologist

Earl Collum, MD        William Anderson, MD        Fran Hahn, MD        Jeff Oliver, MD        Jennifer Eschbacher, MD

Molecular Diagnostics; St. Joseph's Hospital and Medical Center: CLIA # 03D2066951
DATA AND CALCULATION OF DECISIONDX-EC RESULT GENERATION

The DecisionDX-EC assay determines a labeling index of the cellular compartment localization of NF-κB (Abcam, rabbit polyclonal), GLI1 (Abcam, rabbit polyclonal), and SHH (Abcam, rabbit monoclonal, clone EP1190Y) and were detected with a MACH4 HRP polymer (biotin-free) detection system (Biocare). NF-κB and GLI1 are each scored by determining the presence of nuclear localization in tumor cells compared to total tumor cells. SHH is scored by determining the presence of cytoplasmic localization in tumor cells compared to total tumor cells. These intermediate results are then analyzed using a logistic regression algorithm trained on a set of tumors with known outcomes.

BACKGROUND

Clinical development and clinical validation studies: The DecisionDX-EC assay was discovered and completed clinical development and validation under research laboratory conditions at MD Anderson Cancer Center (MDACC). Data from these initial development and validation studies included patients who received pre-operative chemoradiation (CTRT) followed by surgery. For these studies, researchers defined extreme resistance to chemoradiation therapy as patients having greater than 50% residual tumor upon surgical resection per the Rohatgi research tool. In the independent clinical validation study of 167 patients, the logistic regression model used to classify extreme resistance samples yielded an uncorrected area under the receiver operating curve, or AUC, of 0.96 and corrected AUC of 0.95 (Ajani, 2011). Castle Biosciences exclusively licensed the DecisionDX-EC technology and completed separate technical analytic and clinical validation studies.

Castle Biosciences analytic validation studies: Employing over 300 specimens, CAP accredited / CLIA certified technical SOPs were developed. The SOPs were technically validated in a second, third-party contracted laboratory. Concurrent blinded scoring was also undertaken by two clinical experts, a pathologist and a doctorate-level clinical scientist. These two experts achieved 97% concordance with kappa=0.92 (95% CI=0.81-1.00).

Clinical validation study #2: Another objective of clinical validation study #2 was to establish the concordance between the research grading tool used by MDACC (Rohatgi, et al) and the College of American Pathologists (CAP) Treatment Response Grading (TRG) tool used clinically for esophageal cancer. This study employed centralized blinded pathology scoring of paired post-treatment resection tissue with results as follows:

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CLINICAL EXPERIENCE

Clinical validation #2 study: A second, prospectively planned multi-center clinical validation study was initiated and completed following finalization of CAP accredited / CLIA certified standard operating procedures.

This study included 64 patients all of whom received pre-operative CTRT and of whom 67% had evidence of 5-FU based pre-operative CT regimes. The following statistics were obtained from the submitted pre-operative, pre-CTRT biopsy specimens by a clinical scientist who was blinded to the pathologic outcomes of the CTRT therapy.

- AUC: 0.96
- Accuracy %: 84%
- Specificity %: 95%
- Sensitivity (%): 64%
- PPV (%): 88%
- NPV (%): 83%

All post-CTRT, post-operative surgical specimens were graded according to the CAP treatment response guidelines by an independent, centralized pathologist who was also blinded to the DecisionDX-EC assay result as well as administration, if any, of pre-operative CTRT.

REFERENCE LIST


Molecular Diagnostics; St. Joseph’s Hospital and Medical Center: CLIA # 03D2066951

This test was developed and its performance characteristics determined by Castle Biosciences Inc. and the Molecular Medicine Laboratory of St. Joseph’s Hospital and Medical Center under a master services agreement. Testing was performed at DNA Diagnostics Laboratories, St. Joseph’s Hospital and medical Center, 124 W. Thomas Road, Suite 200, Phoenix, AZ 85013, (602) 406-3104. The laboratory is regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing. This test not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research only. The results are adjunctive to the ordering physician’s workup and should be interpreted in the context of other procedures and diagnostic criteria. Patent Pending.
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### RESULTS

**DecisionDx-EC = Responder**

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