Development of Claudin 18.2 IHC 14G11 pharmDx as a Clinical Trial Assay for the clinical development of anti-Claudin 18.2 monoclonal antibody Osemitamab (TST001) in gastric and gastroesophageal junction adenocarcinoma

Hayley Alderson[1], Katie Lefley[1], Lesly Fregoso[1], Achintya Bharadwaj[1], Dan Castellanos-McKenney[1], Siena Tabuena-Frolli[1], Donna Kell[1], Xinlai Yao[2], Xueming Qian[2], Li Xu[3], Caroline Germa[3], Emin Oroudjev[1]

[1]Agilent Technologies, Inc. [2]Suzhou Transcenta Therapeutics Co., Limited [3]Transcenta Therapeutics Inc.

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Introduction

Claudin-18 isoform 2 (Claudin 18.2) belongs to the human claudin family of tetraspan membrane proteins that are crucial structural and functional components of tight junctions. Unlike other family members, Claudin 18.2 is a tissue-restricted marker that is expressed only in differentiated epithelial cells of the gastric mucosa.[1] In cancer, Claudin 18.2 is ectopically expressed at a significant level in multiple tumor types.[1,2] Claudin 18.2 acts as a cell membrane surface protein with an exposed extracellular structure allowing antibody binding and it is suggested that Claudin 18.2 can be an ideal target for the development of therapeutic monoclonal antibodies.

A mouse anti-Claudin 18.2 monoclonal antibody, clone 14G11, was developed by Transcenta. The antibody was generated against a linear epitope located on the extracellular domain of loop1 and has a binding site that overlaps with the binding site of therapeutic antibody Osemitamab. It was selected by Transcenta to develop an IHC assay in collaboration with Agilent Technologies, Inc.

Agilent Technologies, Inc. is developing Claudin 18.2 IHC 14G11 pharmDx as an immunohistochemical (IHC) assay for the detection of Claudin 18.2 protein in gastric and gastroesophageal junction (GEJ) adenocarcinoma in support of clinical studies conducted by Transcenta Therapeutics.

Methods

This IHC assay is based on EnVision FLEX visualization technology using Monoclonal Mouse Anti-Human Claudin 18.2, Clone 14G11 primary antibody. The assay staining protocol was developed for Dako PT Link and Autostainer Link 48 automated IHC platform through extensive optimization testing. The assay includes Claudin 18.2 primary antibody and Negative Control Reagent (NCR), which are used in conjunction with ancillary reagents.

Analytical verification for Claudin 18.2 IHC 14G11 pharmDx included sensitivity, specificity, precision, and robustness assessment. Unless otherwise noted, testing was performed on commercially-procured human formalin-fixed, paraffin-embedded (FFPE) gastric and GEJ adenocarcinoma tissues. Protein expression was evaluated using Tumor Proportion Score (TPS). The TPS is defined as the percentage of viable invasive tumor cells showing partial or complete Claudin 18.2 membrane staining at any intensity.

Results

Sensitivity

The assay detects the Claudin 18.2 protein in gastric/GEJ adenocarcinoma tissues. A range of Claudin 18.2 expression from TPS 0% – 100% was observed in 101 gastric/GEJ FFPE specimens.

Results continued

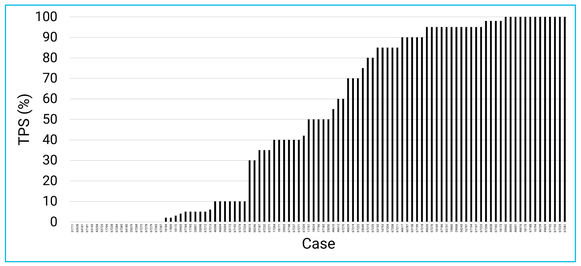

Figure 1. Sensitivity in gastric/GEJ adenocarcinoma.

Specificity

Staining

The assay shows crisp membrane staining of tumor cells and minimal to no cytoplasmic staining.

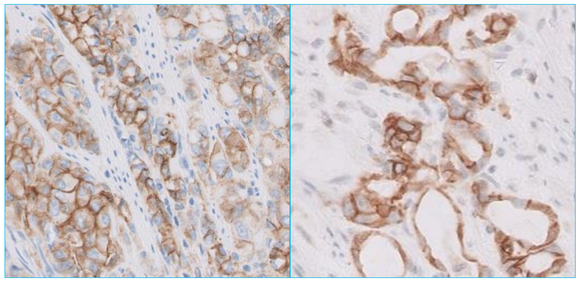

Figure 2. Tumor membrane staining in gastric adenocarcinoma.

Peptide Inhibition

Testing with recombinant Claudin 18.2 protein and Claudin 18.1 (Claudin 18 isoform 1) protein shows Monoclonal Mouse Anti-Human Claudin 18.2, Clone 14G11 selectively binds with Claudin 18.2.

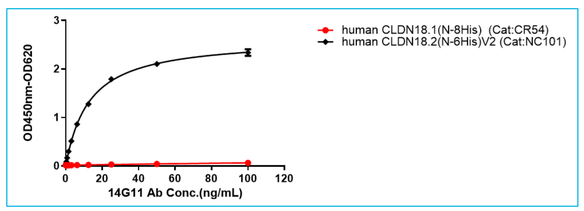

Figure 3. Peptide inhibition with Claudin 18.1 and Claudin 18.2.

Normal Tissue

Membrane staining was observed in 2 out of 31 normal tissues tested: stomach and tonsil. Strong membrane staining was present in gastric glandular mucosa of stomach tissue. Notably, no membrane staining was observed in lung tissues, where Claudin 18.1 is expected to be expressed, thus demonstrating antibody specificity to Claudin 18.2. In tonsil tissue, weak staining of the crypt epithelium was noted.

Results continued

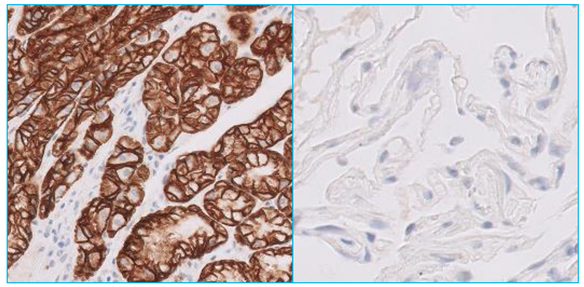

Figure 4. Claudin 18.2-stained normal stomach (left) and normal lung (right) tissues.

Tumor Tissue

A total of 33 non-gastric/GEJ tumor types (118 specimens) were tested. Positive Claudin 18.2 reactivity was observed in pancreatic adenocarcinoma (3 out of 6 specimens tested) and bronchoalveolar carcinoma (2 out of 4 specimens tested), which is consistent with expected Claudin 18.2 expression in these malignancies.[3,4]

Precision and Robustness

Observer Precision

To assess the inter-observer and intra-observer precision, 78 gastric/GEJ adenocarcinoma specimens were tested and evaluated by three pathologists, each performing three independent reads. Negative percent agreement (NPA), positive percent agreement (PPA), and overall percent agreement (OA) was calculated for inter-observer precision and intra-observer precision based on two TPS cutoff values. A subset of 60 specimens were analyzed for each cutoff.

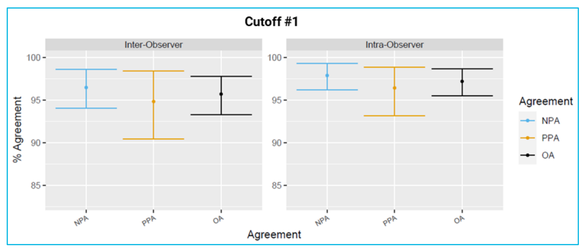

Figure 5. Inter- and intra-observer precision, cutoff #1.

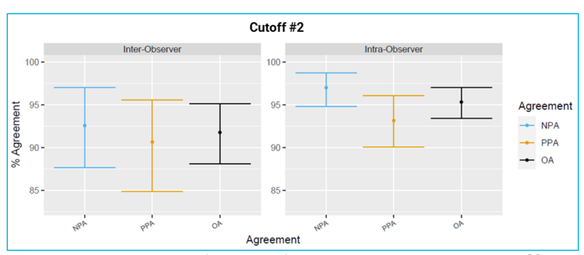

Figure 6. Inter- and intra-observer precision, cutoff #2.

Results continued

Laboratory Precision and Robustness

Laboratory precision and robustness evaluated the conditions listed in Tables 1 and 2, respectively. A minimum of six gastric/GEJ adenocarcinoma specimens were used in each sub-study. Differences in TPS scores across conditions were compared to the expected assay variability based on historical knowledge and early assay development. Results showed acceptable staining variability for each sub-study.

Table 1. Laboratory precision.

Sub-study	Conditions	% of TPS differences within expected variability
Intra-run	Five replicates within the same run	100.0%
Inter-day/run	Three non-consecutive days/runs	100.0%
Inter-instrument	Three instruments	96.3%
Inter-operator	Three operators	95.7%
Inter-lot	Three reagent lots	95.1%

Table 2. Robustness.

Sub-study	Conditions	% of TPS differences within expected variability
Target Retrieval Time	18–22 minutes	100.0%
Target Retrieval Temperature	95–99 °C	100.0%
Target Retrieval pH	8.7–9.2	98.1–100.0%
Target Retrieval Re-use	1st and 3rd use	100.0%
Tissue Thickness	3–5 µM	96.3–100%
Slide Type	FLEX IHC and Superfrost Plus	100.0%
DAB Chromogen Concentration	18–22 drops	96.3–100%
Overnight Run Tolerance	Day and overnight	100.0%

Conclusions

Analytical verification testing indicates Claudin 18.2 IHC 14G11 pharmDx is a sensitive, specific, precise, and robust assay for detecting Claudin 18.2 in gastric and GEJ adenocarcinoma. Claudin 18.2 IHC 14G11 pharmDx for Investigational Use Only/for Performance Evaluation Only will be used for patient selection in the phase III trial of gastric/GEJ adenocarcinoma where applicable ethics committee and regulatory approvals have been granted.

References & Acknowledgments

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Tissue samples supplied by BioIVT (Hicksville, NY, USA)