Development and Validation of a Biomarker For Prospective Selection of NOTCH1 Activation in Patients with Certain Advanced Solid Tumors in A First-in-human Phase1 Study of the Cancer Stem Cell Targeting Antibody OMP-52M51 (anti-NOTCH1)

Belinda Cancilla1, Raymond Tam1, Chun Zhang1, Steve Anderson2, John Lewicki1, Tim Hoey1, Bryan McCune1, Lori Johnson2, Esdoe Iudusogie1, Ann M. Kapoun1

1OncoMed Pharmaceuticals, California; 2LabCorp, Research Triangle Park, North Carolina

Abstract

The NOTCH pathway plays a key role in embryonic development, the regulation of stem and progenitor cells, and is implicated centrally in many forms of human cancer. NOTCH1 is known to be frequently activated in certain solid tumor types. OMP-52M51 is a humanized IgG2 antibody that inhibits the signaling function of the NOTCH1 receptor. Mouse xenograft studies using missense-mutated, patient-derived xenografts have shown that OMP-52M51 impedes tumor growth and selectively eliminates cancer stem cells in a range of tumor types particularly in tumors with activated NOTCH1 signaling.

We previously reported the frequency of NOTCH pathway activation across a large panel of human tumors (n=800) by an immunohistochemistry (IHC) assay that detects the activated form of the NOTCH1 using an antibody that specifically recognizes the NOTCH1 intracellular domain (ICD). Using this test and a rigorous H-score cut-off, we found elevated NOTCH1/ICD in 73% of the following cancers: chemo-resistant breast (29%), gastric (13%), esophageal (27%), hepatocellular (26%), cholangiocarcinoma (20%), ovarian (25%), kidney (12%), bladder (13%), lung (8%), and colorectal cancer (5%). Here we developed a specific CLIA-validated IHC assay capable of identifying patients with activated NOTCH1 signaling. The assay was transferred to LabCorp for testing and validation. Interpretation of results obtained by OncoMed and LabCorp demonstrated a correlation of >99%.

Methods

An IHC staining protocol was developed by OncoMed for anti-NOTCH1 ICD ( Cleaved NOTCH1, Val1744) on formalin-fixed, paraffin embedded FFPE tissues using a Ventana Ultra Automated stainer. The assay was transferred to LabCorp for testing and validation. Interpretation of NOTCH1 ICD staining was performed by a board-certified, anatomic pathologist using conventional light microscopy at LabCorp, compared to semi-automated tissue analysis using the Aperio (Leica) analysis platform. Staining was assessed using the H-score format, recording both the intensity of staining (0-no staining, 1-weak staining, 2=moderate staining and 3=strong staining) and percentage of tumor cells staining (0-100%).

H-Score = 3 (3+ positive nuclear %) + (2 x (2+ positive nuclear %)) + (1 x (1+ positive nuclear %))

The following assay parameters were evaluated in the method validation: accuracy, sensitivity, specificity, precision and prediction. Intra- and inter-assay precision were as well as for inter-operator and inter-observer precision evaluated.

CLIA Assay Validation: Sensitivity

The sensitivity of the IHC method was determined as a function of Patient Derived Xenograft (PDX) tumor tissue with known levels of NOTCH1 expression and therapeutic response data. A total of 22 xenograft tissues were evaluated for NOTCH1/ICD staining by independent staining and analysis at OncoMed and LabCorp.

CLIA Assay Validation: Specificity

We developed a specific CLIA-validated IHC assay capable of identifying patients with activated NOTCH1 signaling. The assay was transferred to LabCorp for testing and validation. Interpretation of results obtained by OncoMed and LabCorp demonstrated a correlation of >99%.

CLIA Assay Validation: Accuracy

The following assay parameters were evaluated in the method validation: accuracy, sensitivity, specificity, precision and prediction. Intra- and inter-assay precision were as well as for inter-operator and inter-observer evaluation.

Summary

A specific CLIA-validated NOTCH1.ICD IHC assay was developed that detects the activated form of NOTCH1:

- Assay was transferred from OncoMed to LabCorp, demonstrating comparable performance
- Accuracy: demonstrated through blinded evaluation of a set of human tumors with varying levels of NOTCH1.ICD
- Sensitivity: confirmed by comparing PDX samples with known levels of NOTCH1.ICD expression and response to therapy
- Specificity: normal human tissues had the expected patterns of staining
- Reproducibility: demonstrated robustness and reproducibility of the assay across replicate human tumor sections, assays, days, operators and interpretation by different pathologists

From this evaluation an H-score cut-off was set to distinguish potential response from non-response in FFPE tumor samples