NOTCH3 expression is predictive of efficacy in pancreas tumor models treated with OMP-59R5, a monoclonal antibody targeting the NOTCH2 and NOTCH3 receptors

Belinda Cancilla, Wan-Ching Yen, Chun Zhang, Marcus M. Fischer, May Ji, Tracy Tang, Yu-Wang Liu, Raymond S. Tam, Min Wang, Austin Gurney, Timothy Hoey, John Lewicki, Ann M. Kapoun
OncoMed Pharmaceuticals Inc., Redwood City, CA 94063

Abstract

The NOTCH signaling pathway regulates key functions during embryonic development, stem cell maintenance and differentiation in adult tissues, and is implicated in many human cancers. OMP-59R5 is a ligand-blocking antibody targeting both the NOTCH2 and NOTCH3 receptors. We have developed a series of primary human xenograft models from patients with pancreatic cancer and used these models to examine efficacy response to OMP-59R5. We found that anti-NOTCH2/3, either as a single agent or in combination with chemotherapeutic agents, was efficacious in pancreatic tumor models.

Expression of NOTCH3 mRNA by next-generation sequencing in ten baseline pancreatic tumors correlates with response to OMP-59R5, where growth of tumors with moderate to high expression of NOTCH3 was significantly reduced compared to tumors with low expression.

We developed a Research-Use-Only (RUO) qPCR assay for measuring NOTCH3 mRNA expression using Formalin-Fixed, Paraffin-Embedded (FFPE) samples. This assay shows consistent correlation with the next-generation sequencing data in the ten pancreatic xenograft tumors.

Expression levels of NOTCH3 were also examined in ~120 human metastatic pancreatic specimens to determine the reportable range of the assay and to identify association with clinical factors. This analysis showed that NOTCH3 gene expression maintained the same distribution across different specimen types, such as biopsy, surgical biopsy and surgical resection, etc. Samples with clinically relevant sites of recurrence also showed a similar range in NOTCH3 gene expression.

Moreover, we developed an immunohistochemistry (IHC) assay for NOTCH3 protein expression. The correlation between the IHC assay and the qPCR assay was examined in both the metastatic pancreatic human specimens and the primary human xenograft xenograft models. A significant correlation was found between the gene and protein levels, suggesting that both NOTCH3 gene expression and protein expression may predict the response to OMP-59R5 in pancreatic cancer.

We are evaluating NOTCH levels and patient response in ALPINE, a Phase 1b/2 anti-NOTCH2/3 trial in first-line advanced pancreatic cancer patients.

Methods

Correlation of NOTCH3 gene expression with anti-NOTCH2/3 efficacy was examined by using minimally passaged patient derived pancreas tumor xenograft models.

qPCR assays were designed using hydrolysis probe chemistry for NOTCH3. Nine assays were designed in total using the NOTCH3 REFSEQ (NM_000435.2). The best performing assay was selected through assessment of efficiency, specificity and linearity for all the 9 assays. Matched fresh frozen (FF) and the formalin-fixed paraffin embedded (FFPE) xenograft samples were also used to examine the performance of the assays.

ANOVA was used to test the association of the delta cq with technical and clinical factors, including patient age, sample age, specimen type, site of recurrence, sex and sample vendor in ~120 metastatic pancreatic human tumor samples.

NOTCH2/3 IHC assay was developed using an in-house murine monoclonal antibody specific for the extracellular domain of human NOTCH3.

Correlation of the IHC assay and the qPCR expression was examined in both xenograft and human pancreas tumor samples.

Results

Anti-NOTCH2/3 Efficacy in Pancreatic Xenograft Tumor Models

Correlation Between Anti-NOTCH2/3 Tumor Inhibition and Levels of Tumor-expressed NOTCH3

Pancreatic Cancer NOTCH3 Gene Expression: Human Liver and Lymph Metastatic Tissues & Primary Xenograft Tumor Models

Correlation between NOTCH3 Gene Expression (RUO Assay) and Protein (IHC Assay)

Summary

Significant correlation was found between the levels of tumor-derived NOTCH3 and the efficacy of Anti-NOTCH2/3 in patient derived primary xenograft models.

Developed a Research-Use-Only (RUO) qPCR assay for measuring NOTCH3 gene expression in Formalin-Fixed, Paraffin-Embedded (FFPE) samples.

Developed an immunohistochemistry (IHC) assay for NOTCH3 protein expression. The NOTCH3 IHC assay showed good correlation with the RUO gene assay in pancreas tumors.

The RUO assay is being used to evaluate NOTCH3 levels and patient response in ALPINE, a Phase 1b/2 Anti-NOTCH2/3 trial in first-line advanced pancreatic cancer patients.