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

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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 *NOTCH3* expression data 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 pancreatic 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 NOTCH3 levels and patient response in ALPINE, a Ph1b/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 pancreas human tumor samples.

NOTCH3 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.

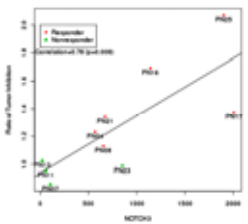
Results

Anti-NOTCH2/3 Efficacy in Pancreatic Xenograft Tumor Models

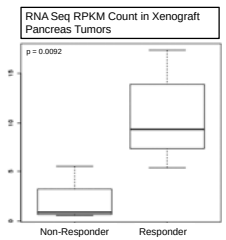
Pancreas Tumor Model	Efficacy (Combined With Gemcitabine)
PN04	+
PN08	+
PN16	+
PN17	+
PN21	+
PN25	+
PN07	–
PN11	–
PN13	–
PN23	–

*p<0.05 vs. Gem as a single agent

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



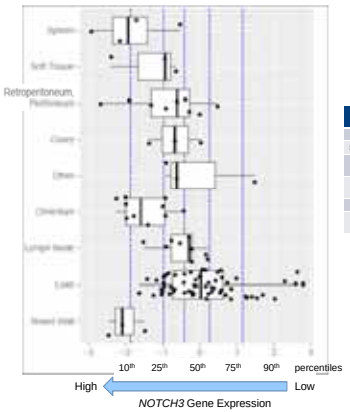
Tumor-expressed *NOTCH3* in Responder vs. Non-responder Xenografts



10 primary patient-derived pancreatic tumor xenografts were tested for efficacy in response to Anti-NOTCH2/3 (OMP-59R5) plus Gemcitabine.

Significant correlation was found between the levels of tumor-derived *NOTCH3* and the efficacy of Anti-NOTCH2/3. Responder tumors have higher levels of NOTCH3 compared to non-responders when treated with Anti-NOTCH2/3 plus Gemcitabine.

NOTCH3 Gene Expression in Pancreatic Cancer Metastatic Tissues

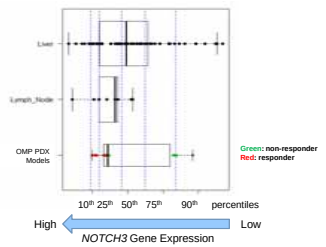


	d.f.	Partial SS	MS	F	P
PatientAge	1	0.001	0.001	0.001	0.9771
Sample Age	1	3.021	3.021	3.213	0.0767
Specimen Type	3	4.862	1.621	1.724	0.1686
Site of Recurrence	8	33.071	4.134	4.397	0.0002
Sex	1	0.437	0.137	0.465	0.4974
Sample Vendor	5	6.128	1.226	1.304	0.2706

ANOVA showing statistical significance of variables retained in the fitted model

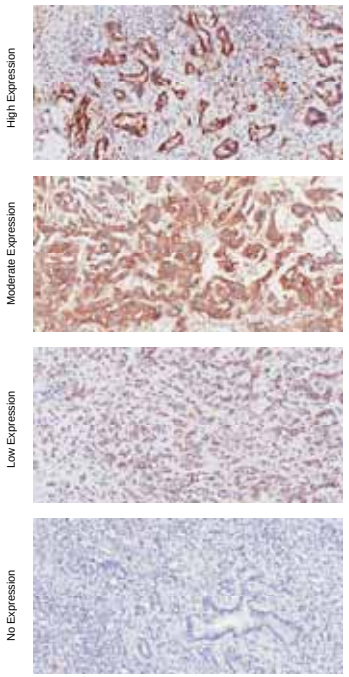
NOTCH3 gene expression RUO RT-PCR assay in sourced tumor metastatic tissues from first line pancreatic cancer patients (n=~100 samples) to obtain *NOTCH3* gene threshold and incidence of *NOTCH3* high expression.

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



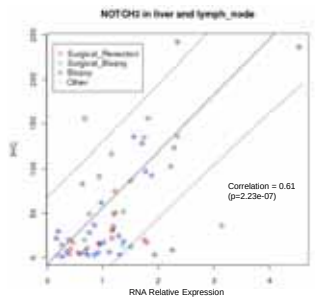
Normalized Distribution of 69 Metastatic Pancreas Human Liver and Lymph Node Samples and Pancreas Xenograft Tumor Models

Pancreatic Cancer NOTCH3 IHC Expression in Liver and Lymph Node Metastatic Tissues

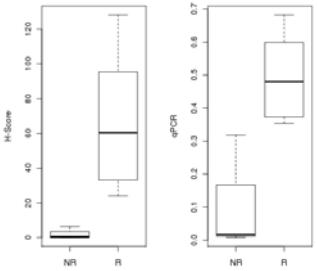


Correlation between *NOTCH3* Gene Expression (RUO Assay) and Protein (IHC Assay)

In the linear range: Correlation = 0.82 (p=1.765e-13)



Both IHC and qPCR Showed Clear Separation Between Responders vs. Non-responders



Calculated relative expression using $2^{-deltaCt}$ method

Summary

Significant correlation was found between the levels of tumor-derived *NOTCH3* and the efficacy of Anti-NOTCH2/3 in patient derived primary tumor 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 Ph1b/2 Anti-NOTCH2/3 trial in first-line advanced pancreatic cancer patients.