Monoclonal antibodies to Notch receptors inhibit tumor maintenance

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Abstract (#5170)

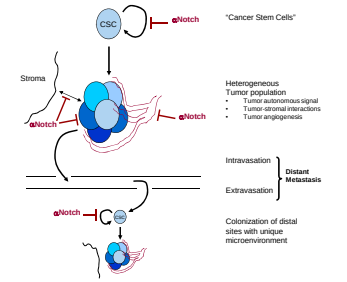
AVEO has developed a series of inducible mouse models of cancer which, through the preservation of critical tumor/stromal interactions, facilitate identification of cell-surface and secreted proteins that represent viable targets for therapeutic antibodies and other biologics. Functional genetic screens performed *in vivo* in these models identified the Notch pathway as a critical regulator of tumor maintenance. This finding is consistent with emerging evidence that activation of Notch signaling via receptor point mutation, receptor amplification, and elevated receptor and ligand expression, plays a key role in various human cancers. Moreover, the Notch pathway controls diverse aspects of tumorigenesis and tumor maintenance, regulating tumor autonomous processes and interactions with the microenvironment, including angiogenesis. To further understand the role of the Notch pathway in tumor maintenance, and to assess the therapeutic potential of targeting the Notch pathway in cancer, we have generated monoclonal antibodies that inhibit various Notch receptors.

Characterization of a Notch1-specific monoclonal antibody through cell-based and biochemical studies demonstrated that the antibody bound to the Notch1 ligand binding domain with high affinity, prevented ligand mediated activation of the receptor, and specifically repressed Notch1-dependent signaling with high potency. Mice treated with the Notch1 antibody exhibited altered T cell fate specification as expected for loss of Notch1 function. Monoclonal antibody inhibition of Notch1 could be effectively combined with other therapies to enhance anti-angiogenic effects, or to overcome resistance to VEGF/VEGFR inhibition.

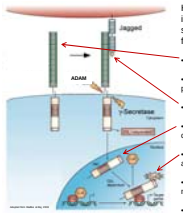
To identify tumors that are dependent upon tumor autonomous Notch signaling the expression of Notch pathway related genes was correlated with Notch pathway dependence in human cancer cell lines. Active Notch signaling alone did not predict dependence upon Notch, but expression of a single Notch target gene, HeyL, was highly correlated with sensitivity of human cancer cell lines to inhibition of ligand-dependent Notch signal. The utility of this biomarker was further confirmed by the identification of a subset of Kras mutant pancreatic and colon cancer cell lines that were subsequently demonstrated to be highly sensitive to Notch pathway inhibition. Hence, HeyL expression may serve as a predictive biomarker of Notch-dependent tumors.

These data support the clinical development of AVEO's humanized Notch antibodies for the treatment of human cancer.

Notch in Cancer



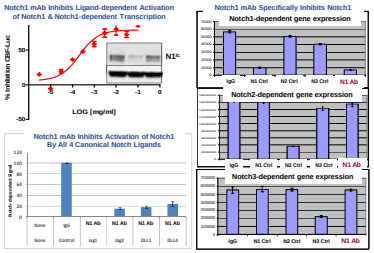
Identification of Notch1 Inhibitory Antibodies



Hybridomas generated from mice immunized with Notch1 ECD were screened and filtered using the following assays:

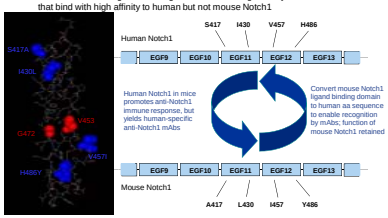
- Binding to human Notch1 on the cell surface
- Binding to recombinant human Notch1 protein
- Neutralization of ligand binding to Notch1
- Inhibition of Notch1 cleavage and intra-cellular domain release
- Inhibition of Notch1-dependent reporter activity
- Activation of HUVEC branching morphogenesis *in vitro*
- Inhibition of T-ALL cell proliferation *in vitro*

Notch1 mAb Inhibits Notch1-dependent signaling

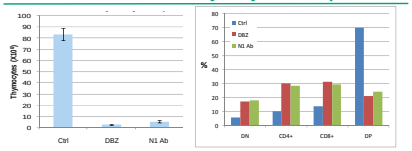


Humanization of Mouse Notch1

The mouse Notch1 gene was engineered to be recognized by Notch1 mAbs that bind with high affinity to human but not mouse Notch1



Notch1 mAb Inhibits Thymocyte Development



Notch mAbs Inhibit Ligand-dependent signaling

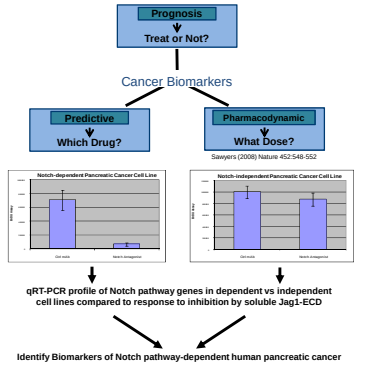
Inhibitors of Ligand-dependent activation of Notch receptors

	Notch1 mAb	Notch2 mAb	Notch3 mAb
Binding to Human Notch	Yes	Yes	Yes
Binding to Mouse Notch	No[#]	Yes	No
Neutralization of Ligand binding	Yes	Yes	Yes
Inhibition of Notch-dependent signaling	Yes	Yes	Yes
Activity *in vivo*	Yes	Yes	Yes
Humanized Development Candidate	In progress	tbd	In progress

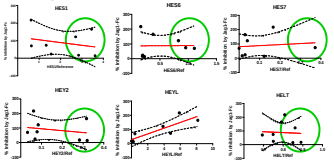
[#] Notch1 has been humanized in mice to enable targeting of host-dependent processes

Notch1 → Angiogenesis is likely to be a viable target
How do we identify tumors that are dependent upon Notch?

Biomarkers & The Genetic Context of Notch Dependence

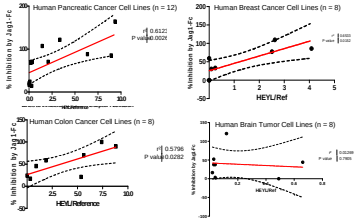


Correlation of Notch Target Gene Expression with Notch-dependence in Pancreatic Cancer Cell Lines



- **Notch pathway activity does not predict Notch pathway dependence**
- **Expression of HeyL appears to correlate with Notch pathway-dependence**

HeyL Expression Correlates with Notch-dependence in Specific Cancer Settings



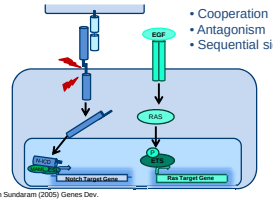
HeyL May Correlate with Dependence Upon Ligand-dependent Notch Signaling

- T-ALL lines with ligand-independent Notch1 mutations do not express elevated HEYL
- T-ALL lines with ligand dependent Notch1 signaling may express elevated HEYL

Cell Line	Notch1 Mutation Status: HD Domain	Notch1 Mutation Status: Pest Domain	Ligand dependent Notch1 Signal	FBXW7 Mutation	PTEN Status	% Inhibition by GSI	% Inhibition by slag1-Fc	HEYL/Ref
ALL SIL	L1594P	Y	N	wt		> 100%	0	0.026
DND41	L1594P/D1610V	Y	N	wt		> 100%	16.9	0.026
HPB ALL	L1575P	Y	N	wt		> 100%	18.7	0.039
HSB-2	wt	N	Y	R505C		54.4	53.6	2.305
KarpasAS	wt	Y	Y	COSMIC		75.0	78.3	2.222
	L1710P	N						
T-ALL1	wt	N	Y	wt		> 100%	97.4	0.134

- HEYL may indicate dependence upon ligand-dependent Notch signaling
- Additional information beyond HEYL status is important in T-ALL
  e.g. Fbxw7 status, PTEN expression, expression of other Notch targets (O'Neill (2007) , Palomero (2007), Rao (2009))

Interaction of the Ras and Notch Pathways



- Cooperation
- Antagonism
- Sequential signaling

Adapted from Sundaram (2005) Genes Dev.

Identification of a Subset of Kras Tumors that are Notch-dependent

1. Kras wt cell lines appear to be less dependent upon Notch
   - (Colo205, HT29, Hs766T, BxPC3)
2. A subset of Kras mutant cell lines are Notch-dependent
3. Can identify Notch-dependent Kras mutant cell lines by elevated HEYL expression

Colon	Jag1-Fc Inhibition	Kras Status	HEYL (Cells/HuRef)
LS1034	90%	mut	8
SW480	90%	mut	8.8
HCT116	70%	mut	5.1
HCT-15	58%	mut	1.9
DLD-1	46%	mut	1
Colo205	24%	wt	0.007
HT-29	17%	wt	0.25

Pancreas	Jag1-Fc Inhibition	Kras Status	HEYL (Cells/HuRef)
PANC10.05	217%	mut	6.4
CAPAN-1	165%	mut	9.4
SW1990	122%	mut	3.4
PL45	107%	mut	1.4
PANC-1	74%	mut	8.9
CAPAN-2	72%	mut	2.1
MiaPaCa-2	70%	mut	0.5
HPAF-II	30%	mut	0.2
AsPC-1	20%	mut	0.02
HPAC	17%	mut	0.02
Hs766T	24%	wt	0.1
BxPC-3	14%	wt	0.3

- **A subset of Kras driven cancers may be dependent upon Notch**
- **HeyL could be used to identify Notch-dependent Kras mutant tumors**

Summary

- AVEO has generated Notch-specific monoclonal antibodies that bind specific Notch receptors with high affinity, block ligand-mediated activation, and specifically repress Notch-dependent signaling
- Mice treated with the Notch1 mAb exhibit altered T-cell fate specification as expected for loss of Notch1 function
- This Notch1-specific monoclonal antibody can be further used to explore the role of Notch1 in other aspects of tumor maintenance including roles in angiogenesis and cancer stem cell maintenance
- Detection of active Notch signaling is insufficient to identify tumors dependent upon Notch
- Elevated expression of HeyL may correlate with dependence upon ligand-dependent Notch signaling
- A subset of Kras driven tumors appear to be dependent upon Notch signaling, and elevated expression of HeyL may help identify these tumors
- HeyL plus additional biomarkers of Notch pathway activity may facilitate the identification of patients amenable to therapy targeting the Notch pathway, including AVEO's Notch antibodies

101st American Association for Cancer Research Annual Meeting, Apr. 17-21, 2010, Washington, D.C.