Antitumor Activity of Anti-RON Antibodies and Biomarker of Response

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Abstract

Recepteur d'Origine Nanatais (RON or MST1R) receptor tyrosine kinase is a member of the c-Met RTK family. Macrophage stimulating protein (MSP or MST1) serves as its only known activating ligand. Overexpression of RON has been demonstrated in multiple solid tumor types, and it correlates with disease progression. A potentially oncogenic splicing variant has also been observed in colorectal cancer. The overexpression of RON in lung and breast epithelial cells has been shown to induce tumor development and metastasis in animal models. Inhibition of RON kinase activity via dominant negative receptor, small-molecule inhibitor, and antibodies leads to tumor growth inhibition in several preclinical models. Investigating the antitumor therapeutic potential of an anti-RON antibody is warranted, and a predictive biomarker to guild the therapeutic development is important. A panel of functional anti-RON antibodies with high binding affinity were isolated from murine hybridomas and extensively characterized. Several antagonistic antibodies were identified by their ability to inhibit MSP-induced cell signaling, cell proliferation, migration, and invasion. Several of these antibodies can induce receptor internalization and degradation. The antibodies were also able to inhibit xenograft tumor growth driven by wild-type (WT) RON or the RON delta 160 variant.

We have also identified a multi-gene biomarker to identify tumor lines with potentially activated RON pathway, and it is being validated in a panel of xenograft studies. This signature may also help to predict which tumor types or subtypes are more likely to respond to anti-RON antibody treatment.

Lead murine antibodies 29B06 and 07F01 are humanized, and the humanized derivatives have comparable activities to the parental antibodies.

Acknowledgements

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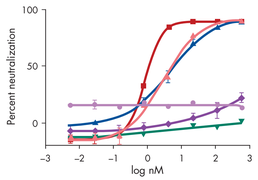
Anti-RON mAbs potently inhibit RON functions in vitro

Anti-RON antibodies potently inhibit MSP binding to RON

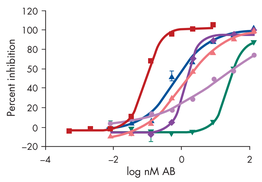
Neutralization of ligand binding



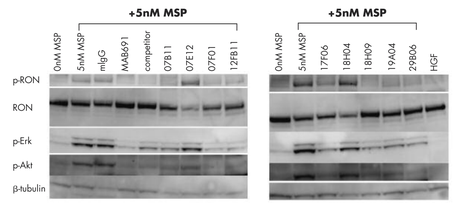
Antibody	EC_{50} (nM)	SD
07F01	0.26	0.05
18H09	0.91	0.15
19A04	non-neutralizer	
29B06	1.11	0.05
12B11	non-neutralizer	
17F06	non-neutralizer	

Anti-RON antibodies inhibit MSP-induced signaling in T47D Cells

p-ERK inhibition



Antibody	IC_{50} (nM)	SD
07F01	0.07	0.02
18H09	0.71	0.36
19A04	15.48	11.53
29B06	0.44	0.27
12B11	5.91	5.92
17F06	0.96	0.4



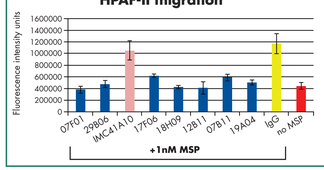
Binding kinetics to RON SEMA+PSI protein by Biacore

Antibody	Measurements at 37°C (SEMA+PSI)			
	ka (1/Ms)	kd (1/s)	K_D (M)	n
07F01	2.00E +06	8.00E –04	4.00E –10	3
29B06	5.20E +05	6.90E –04	1.30E –10	3
17F06	2.60E +05	2.10E –05	1.30E –10	3
18H09	5.80E +05	1.20E –04	2.20E –10	2
12B11	No binding			

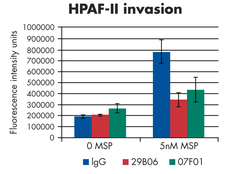
Antibody binding to cell surface RON

Antibody	Cell surface binding by FACS	PC3 (WT RON)	HT-29 (RONΔ160)
29B06	% binding@10μg/ml	99	99
	KD (nM)	0.13	0.48
07F01	% binding@10μg/ml	99	99
	KD (nM)	0.03	0.34
17F06	% binding@10μg/ml	99	99
18H09	% binding@10μg/ml	99	98
12B11	% binding@10μg/ml	95	89
mIgG	% binding@10μg/ml	6	6

Inhibition MSP-stimulated HPAF-II migration

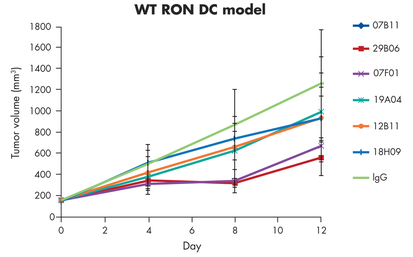


Inhibition of MSP-stimulated HPAF-II invasion

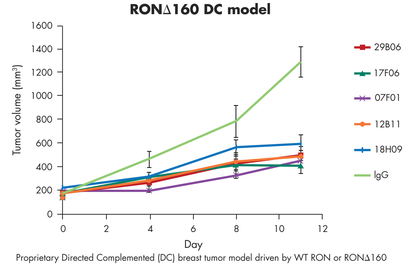


Anti-RON mAbs inhibit RON receptor-driven tumorigenesis

WT RON DC model



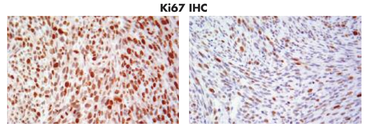
RONΔ160 DC model



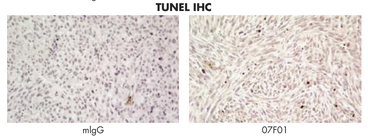
Proprietary Directed Complemented (DC) breast tumor model driven by WT RON or RONΔ160

07F01 decreases proliferation (Ki67) and increases apoptosis (TUNEL) in WT RON DC tumors

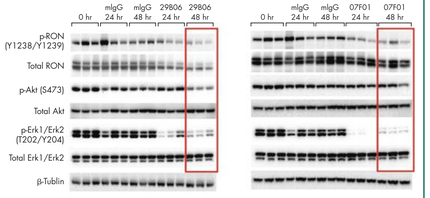
Ki67 IHC



TUNEL IHC



mAbs 07F01 and 29B06 inhibit RON activity and downstream signaling

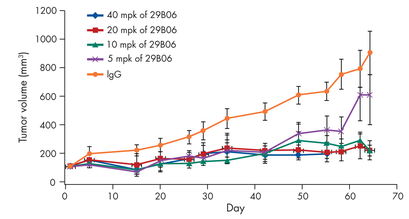


29B06 potently inhibits p-RON, p-Erk, p-Akt, and promotes receptor degradation

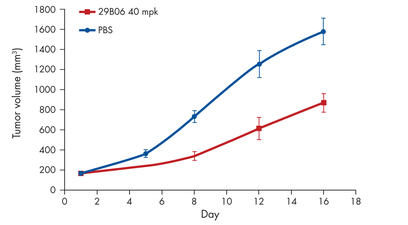
07F01 inhibits p-RON and p-Erk

29B06 is efficacious in human cancer xenografts

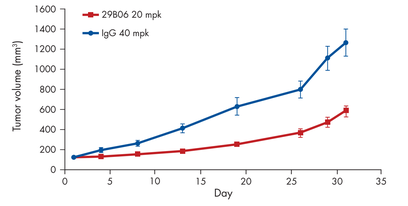
29B06 dose response with H358 xenografts



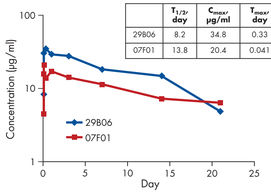
WIDR xenograft–expressing RONΔ160



BxPC-3 xenograft



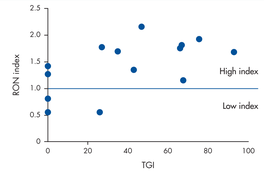
Pharmacokinetic parameters of 29B06 and 07F01



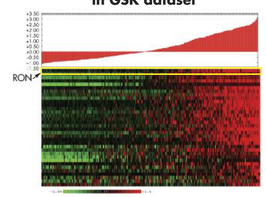
	$T_{1/2}$, day	C_{max}, μg/ml	T_{max}, day	AUC, day•μg/ml	Vd, ml/kg	Cl, ml/day/kg
29B06	8.2	34.8	0.33	421.17	27.9	2.4
07F01	13.8	20.4	0.041	337.41	59.2	3.0

- 29B06 is efficacious at inhibiting tumor growth in more than 10 xenograft models, including tumors expressing WT RON and RONΔ160
- 29B06 treatment decreases proliferation (Ki67 IHC), increases apoptosis (cleaved caspase-3 IHC), decreases angiogenesis (CD31 IHC), and induces receptor degradation (RON western) in xenografts
- 29B06 was administered twice weekly at the indicated doses

Biomarker of 29B06 response

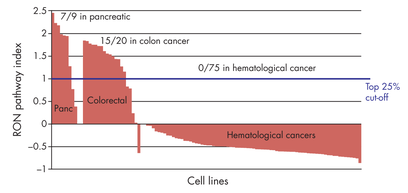


RON index and individual genes in GSK dataset



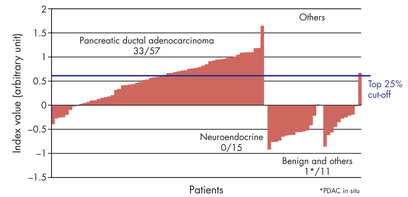
- A gene set consists of the top ~40 most consistently correlated genes, with RON expression identified among 9 different human cancer microarrays
- The RON pathway index is the average of the expression levels of these genes in a given dataset
- To date, 14 cells lines with RON expression (by flow-cytometry) were tested for tumor growth inhibition (TGI, %) by 29B06 in xenograft settings
- There is a significant correlation between RON pathway index and TGI, with R = 0.56, *P* <0.038
- Top 25% of cell lines have a high RON index value of >1 in GSK cell line dataset, top 25% of tumors have high RON index value >0.6 in GeneLogic dataset
- The same cancer types are enriched for high RON index in both cancer cell line and human tumor datasets, suggesting that the cell line validation of the biomarker may be applicable to human tumors

Cell lines with high RON index are highly enriched in certain cancer types
GSK cell line dataset: 316 cell lines, diverse types

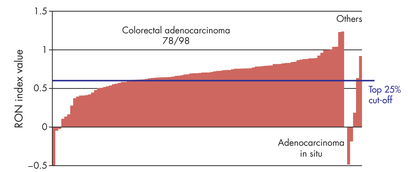


Human tumors with high RON index are highly enriched in certain cancer types
GeneLogic dataset: 660 tumors, 8 types

RON pathway index: pancreatic cancer



RON pathway index: colon cancer



Summary

- **We have identified a panel of high-affinity anti-RON antibodies that inhibit MSP-induced cellular activities, such as p-RON, p-Erk, motility, and invasion**
- **These anti-RON antibodies demonstrated antitumor activity in engineered models driven by RON or RONΔ160, an oncogenic variant of RON**
- **Lead antagonistic antibody, 29B06, demonstrated broad spectrum antitumor activity in a panel of human cancer xenografts**
- **29B06 can induce receptor degradation, decrease RON signaling, decrease proliferation, increase apoptosis, and decrease angiogenesis in xenografts**
- **A RON pathway index biomarker derived from human microarray datasets was shown to correlate with TGI response to 29B06**
- **The same cancer types, such as pancreatic cancer and colorectal cancer, are highly enriched for samples with high RON pathway index in both cell line and human tumor microarray datasets, suggesting the biomarker may be relevant in the clinic if the biomarker is validated in xenograft models**
- **The biomarker may help us identify tumor types or subtypes for clinical investigation of humanized 29B06**

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