Mechanisms of action of a neoantigen-targeting antibody NEO-201

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Abstract

Background: NEO-201 is a humanized IgG1 monoclonal antibody (mAb) that reacts to tumor-associated antigens (TAA) derived from pooled allogeneic colon tumor tissue extracts. NEO-201 recognizes tumor-associated variants of CEACAM family members. This mAb is remarkably tumor-specific in its staining profile and demonstrated its ability to react to wide range of human carcinoma cell lines by flow cytometry and tumor tissues by immunohistochemistry. NEO-201 exhibited both ADCC and complement-dependent cellular cytotoxicity (CDC) activity against human carcinoma cell *in vitro,* and counteracted the growth of human pancreatic xenograft tumors *in vivo*. In this study, we investigated an additional mechanism of action of NEO-201 and to further confirm the rationale for clinical testing using NEO-201 in for treatment of a broad variety of carcinomas. Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is a cell-surface protein expressed by immune cells and tumor cells, and it can inhibit T cell function similar to PD-1 and CTLA-4. CEACAM1 is also a potent inhibitor of natural killer (NK) cell function; binding between CEACAM1 on NK cells and CEACAM1 or CEACAM5 on tumor cells inhibits activation signaling by NKG2D, which prevents NK cell cytolysis and permits tumor cells to evade NK killing.
This study was designed determine whether NEO-201 blocks the CEACAM1 inhibitory pathway to restore antitumor functionality to NK cells.

Methodology: Flow cytometry analysis was used to profile a panel of human carcinoma cell lines for NEO-201 binding. *In vitro* assays, using human tumor cell lines positive for NEO-201, were performed to evaluate NEO-201 ability to mediate ADCC and CDC and to identify CEACAM family members bound by NEO-201. Functional assays were conducted to assess the ability of NEO-201 to potentiate the *in vitro* killing of tumor cells by the NK cell line NK-92, which expresses CEACAM1 and lacks CD16 and the ability to mediate ADCC.

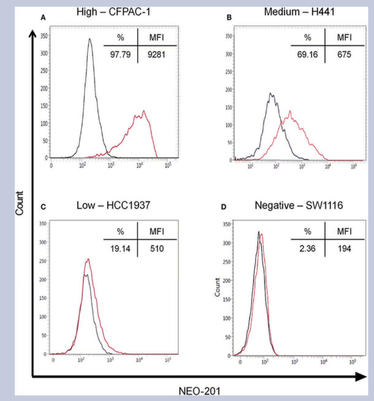
Results: NEO-201 was found to react with a broad range of *in vitro* cultured tumor cell lines. Functional assays revealed that treatment with NEO-201 is capable of mediating both ADCC and CDC against tumor cells expressing the antigen recognized by NEO-201. Expression profiling revealed that various NEO-201$^+$ cell lines expressed different levels of the native forms of CEACAM5/6 vs. the NEO-201-reactive variant forms of these molecules. Functionally, NEO-201 treatment augmented the cytolytic activity of NK-92 cells against NEO-201$^+$ tumor cells in proportion to their level of CEACAM5 expression (average increase of 2-fold), but not against NEO-201$^+$ cells that only expressed CEACAM6.

Conclusions: This study demonstrates that NEO-201 has several mechanisms of action. NEO-201 is able to mediate both ADCC and CDC. In addition, NEO-201 can block the interaction between tumor cell CEACAM5 and NK cell CEACAM1 to reverse CEACAM1-dependent inhibition of NK cytotoxicity. These results suggest that NEO-201 may potentially reverse CEACAM1-dependent immunosuppression of NK cells in patients whose tumors express the NEO-201-reactive variant of CEACAM5.

1. NEO-201 binds to various human carcinoma cell lines

Tumor Cell Line Flow Cytometry

CELL LINE	TUMOR TYPE	% POSITIVE	MFI
COLO 205	**Colon**	**10.33**	**245**
HT-29	**Colon**	**38.40**	**352**
LS174T	**Colon**	**46.46**	**345**
SW1116	Colon	2.36	194
SW1463	Colon	1.23	278
SW480	Colon	1.70	575
ASPC-1	**Pancreatic**	**79.26**	**8927**
BxPC-3	**Pancreatic**	**97.25**	**2584**
CAPAN-2	**Pancreatic**	**29.69**	**327**
CFPAC-1	**Pancreatic**	**97.79**	**9281**
PANC-1	Pancreatic	3.29	289
H441	**NSCLC (adenocarcinoma)**	**69.16**	**675**
H522	NSCLC (adenocarcinoma)	1.38	238
HCC4006	**NSCLC (adenocarcinoma)**	**99.27**	**9899**
HCC827	**NSCLC (adenocarcinoma)**	**77.46**	**692**
SK-LU-1	NSCLC (adenocarcinoma)	1.77	685
CALU-1	NSCLC (squamous)	4.22	571
H1703	NSCLC (squamous)	4.16	111
H226	NSCLC (squamous)	4.83	209
H520	**NSCLC (squamous)**	**61.78**	**443**
AU-565	**Breast (HER2+)**	**50.04**	**227**
BT-474	**Breast (PR+/HER2+)**	**68.79**	**591**
HCC1500	Breast (ER+/PR+)	1.53	597
SK-BR-3	Breast (HER2+)	1.61	329
T-47D	Breast (ER+/PR+)	8.00	161
ZR-75-1	**Breast (ER+/PR+/HER2+)**	**68.80**	**550**
BT-549	Breast (ER-/PR-/HER2-)	1.47	477
HCC1937	**Breast (ER-/PR-/HER2-)**	**19.14**	**510**
HCC38	Breast (ER-/PR-/HER2-)	2.15	226
MDA-MB-468	Breast (ER-/PR-/HER2-)	6.33	344

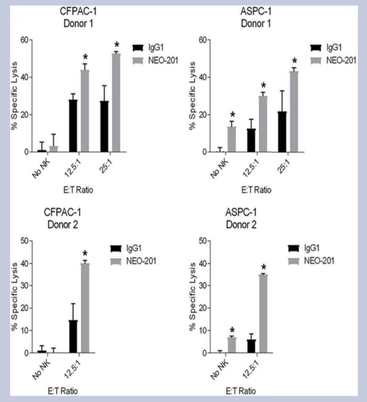


NEO-201 is reactive against a broad range of *in vitro* cultured tumor cell lines. NEO-201 positive cell lines appear in bold text. NEO-201 positivity was defined as % positive >10%.

Positivity was determined using fluorescence minus one (FMO) controls. Positive cell lines were ranked according to their quantified expression level (% positive × MFI), and then sorted into groups of low (<200), medium (200-1000), and high (<1000) expression.

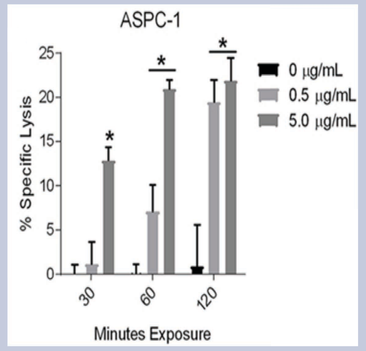
2. NEO-201 mediates ADCC and CDC against human carcinoma cell lines

ADCC assay



ADCC activity using CFPAC-1 or ASPC-1 cells as target cells. Cells were treated with 10μg/mL of NEO-201 or human IgG1 (negative control). Purified NK cells from two healthy donors were used as effector cells at the indicated E:T ratios

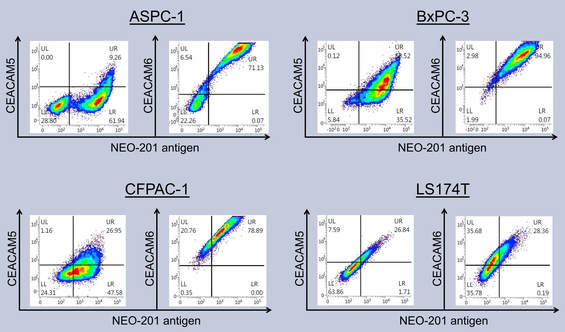
CDC assay



CDC assay using ASPC-1 cells treated with rabbit complement (1:8 dilution) and the indicated doses of NEO-201 for the indicated durations.

3. The NEO-201 antigen is a tumor-associated variant of CEACAM5 and CEACAM6

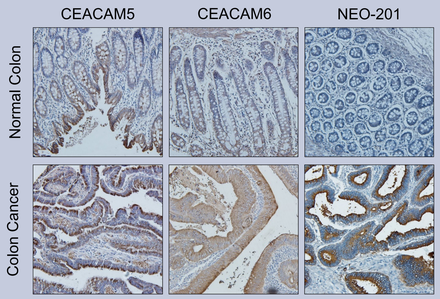
Tumor Cell Line Flow Cytometry



CELL LINE (CANCER TYPE)	MARKER	% POSITIVE	MFI
ASPC-1 (pancreas)	NEO-201	**75.86**	**9,078**
	CEACAM5	20.04	869
	CEACAM6	**77.87**	**52,138**
BxPC-3 (pancreas)	NEO-201	**97.41**	**5,259**
	CEACAM5	**79.54**	**711**
	CEACAM6	**98.45**	**18,690**
CFPAC-1 (pancreas)	NEO-201	**85.21**	**1,728**
	CEACAM5	25.83	1,108
	CEACAM6	**96.50**	**27,792**
LS174T (colon)	NEO-201	29.15	858
	CEACAM5	36.34	1,030
	CEACAM6	**63.41**	**1,462**

Flow cytometry of tumor cell lines discriminates native (NEO-201^{neg}) CEACAM5 and CEACAM6 from the NEO-201-reactive variant forms of CEACAM5 and CEACAM6.

Normal vs. Tumor Tissue Microarray IHC



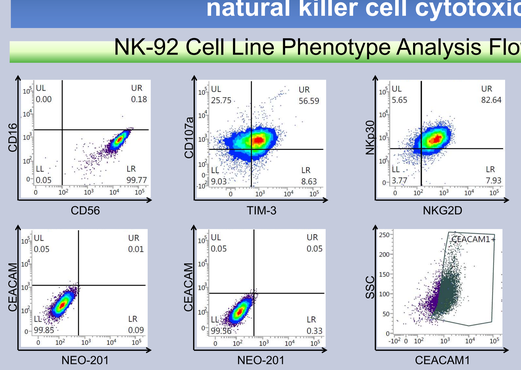
Quantification of Staining

Staining Pattern			Normal Colon (Cases, %)	Colon Cancer (Cases, %)
NEO-201	CEACAM5	CEACAM6		
-	-	-	**2/31 (6%)**	**1/32 (3%)**
+	-	-	0/31 (0%)	0/32 (0%)
-	+	-	0/31 (0%)	0/32 (0%)
-	-	+	**1/31 (3%)**	0/32 (0%)
+	+	-	0/31 (0%)	0/32 (0%)
+	-	+	0/31 (0%)	0/32 (0%)
-	+	+	**28/31 (90%)**	**3/32 (9%)**
+	+	+	0/31 (0%)	**28/32 (88%)**

Similar results observed from normal and cancerous pancreas and lung tissues.

4. The NK-92 cell line is a CEACAM1$^+$ model for non-ADCC natural killer cell cytotoxicity

NK-92 Cell Line Phenotype Analysis Flow Cytometry



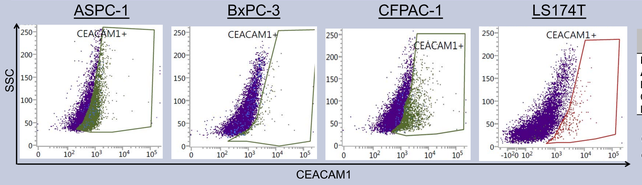
NK-92 cells are an immortalized IL-2-dependent human natural killer cell line that lacks ADCC activity.

NK-92 cells express typical NK cell markers except CD16 (no ADCC):

CD56$^+$	NK lineage marker
CD16^{neg}	ADCC function
CD107a$^+$	Degranulation marker
TIM-3$^+$	Inhibitory receptor
NKp30$^+$	Cytotoxicity receptor
NKG2D$^+$	Cytotoxicity receptor
CEACAM1$^+$	**Inhibitory receptor**

No reactivity with CEACAM5, CEACAM6, or NEO-201 mAb.

Tumor Cell Line CEACAM1 Expression Flow Cytometry

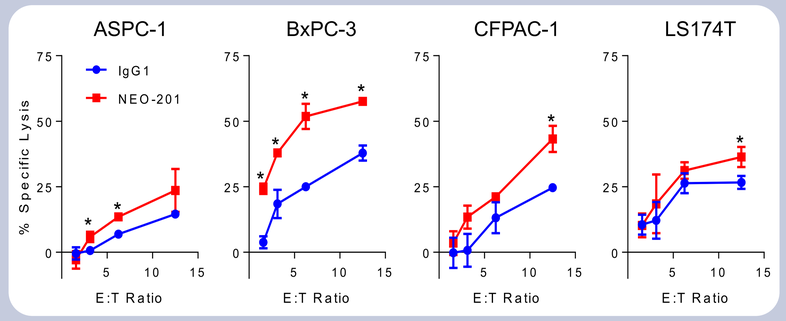


CELL LINE	% POS	MFI
NK-92	**82.57**	**1,270**
ASPC-1	**61.15**	**707**
BxPC-3	2.46	N/A
CFPAC-1	18.67	1,938
LS174T	2.43	N/A

Only ASPC-1 cells highly express CEACAM1.

5. NEO-201 enhances NK-92 cell cytotoxicity against CEACAM5$^+$ / NEO-201$^+$ tumor cells

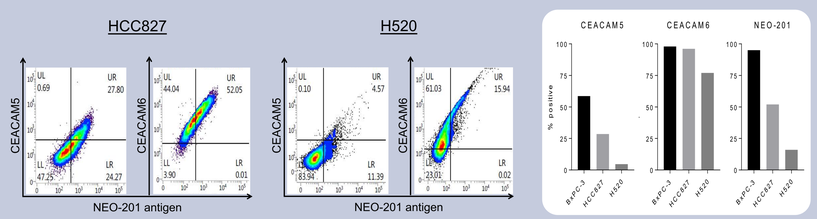
NK-92 16hr Killing Assay -/+ NEO-201 mAb



High NEO-201 antigen Low CEACAM5 High CEACAM1	High NEO-201 antigen High CEACAM5 No CEACAM1	High NEO-201 antigen Low CEACAM5 Low CEACAM1	Low NEO-201 antigen Low CEACAM5 No CEACAM1
Poor enhancement of cytotoxicity	Strong enhancement of cytotoxicity	Some enhancement of cytotoxicity	Poor enhancement of cytotoxicity

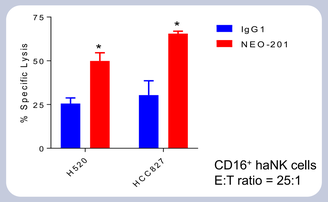
6. NEO-201-mediated enhancement of NK-92 killing depends on tumor cell CEACAM5 variant expression

Tumor Cell Line Flow Cytometry



HCC827 and H520 cells express low CEACAM5 relative to BxPC-3 cells.

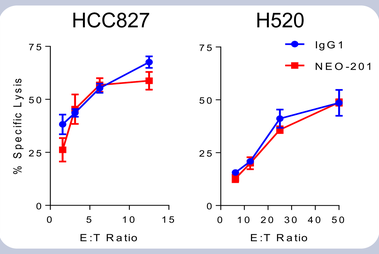
haNK 4hr ADCC Assay



CD16$^+$ haNK cells
E:T ratio = 25:1

NEO-201 mAb is capable of mediating ADCC against HCC827 and H520 cells despite lower levels of NEO-201 antigen.

NK-92 16hr Killing Assay



NEO-201 mAb-mediated enhancement of NK-92 killing does not occur in cell lines that have low expression of CEACAM5.