The neoantigen-targeting antibody NEO-201 enhances NK cell-dependent killing of tumor cells through blockade of the inhibitory CEACAM5/CEACAM1 immune checkpoint pathway

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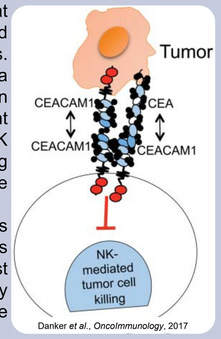
Abstract

Background: Immunotherapy using checkpoint blockade antibodies that target effector cell inhibitory receptors, like PD-1 and CTLA-4, have elicited some dramatic and durable responses in several tumor types. 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.

NEO-201 is a novel humanized IgG1 monoclonal antibody (mAb) that was derived from an immunogenic preparation of tumor-associated antigens (TAAs) from pooled allogeneic colon tumor tissue extracts. It reacts against a wide variety of human carcinoma cell lines and tumor tissues, but is largely non-reactive against normal tissues. NEO-201 binds to members of the CEACAM family, and can activate innate immune mechanisms such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) to kill tumor cells. This investigation was designed determine whether NEO-201 blocks the CEACAM1 inhibitory pathway to restore antitumor functionality to NK cells.



Danker *et al.*, *OncoImmunology*, 2017

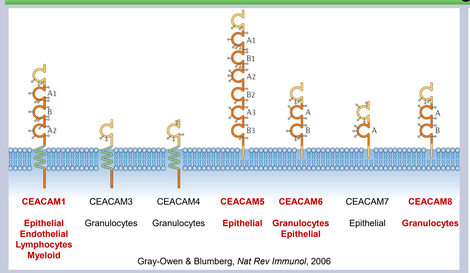
Methodology: *In vitro* assays using human tumor cell lines were conducted to identify CEACAM family members bound by NEO-201. Functional assays were conducted to assess the ability of NEO-201 to potentiate the *in vitro* killing of tumor cells by the NK cell line NK-92, which expresses CEACAM1 and lacks CD16 and the ability to mediate ADCC.

Results: NEO-201 was found to react with distinct variants of CEACAM5 and CEACAM6, but not with CEACAM1 or CEACAM8. Expression profiling revealed that various NEO-201$^+$ cell lines expressed differing 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.

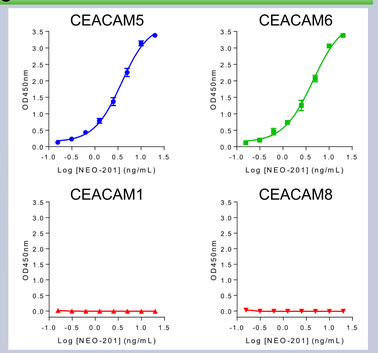
Conclusions: This study demonstrates that NEO-201 is reactive against a tumor-associated variant of CEACAM5/6, and provides evidence that this antibody can block the interaction between tumor cell CEACAM5 and NK cell CEACAM1 to reverse CEACAM1-dependent inhibition of NK cytotoxicity. Experiments are in progress to determine the involvement of NK cell CEACAM1 and/or other checkpoint pathways in this mechanism of action. 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 mAb binds to carcinoembryonic antigen-related cell adhesion molecule (CEACAM) proteins 5 & 6

NEO-201 Binding ELISA

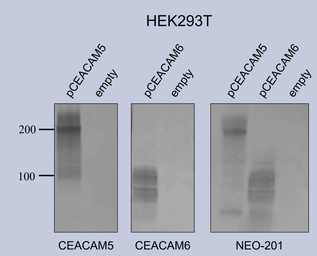


Gray-Owen & Blumberg, *Nat Rev Immunol*, 2006



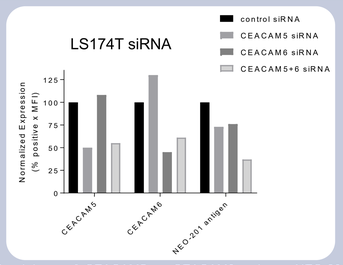
NEO-201 binds to CEACAM5 and CEACAM6 but not to CEACAM1 or CEACAM8.

CEACAM5/6 Overexpression



NEO-201 binds to ectopically overexpressed CEACAM5 and CEACAM6 protein by immunoblot.

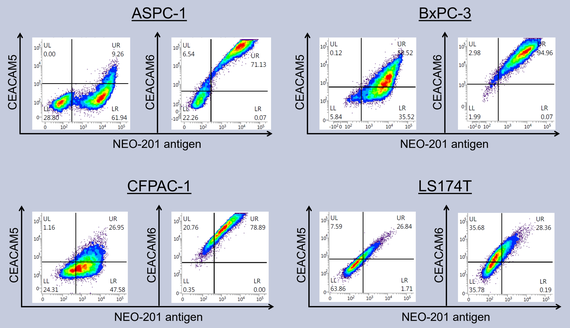
CEACAM5/6 Knockdown



Knockdown of CEACAM5 or CEACAM6 reduces NEO-201 mAb reactivity by flow cytometry. Combination knockdown decreases reactivity greater than single knockdown.

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

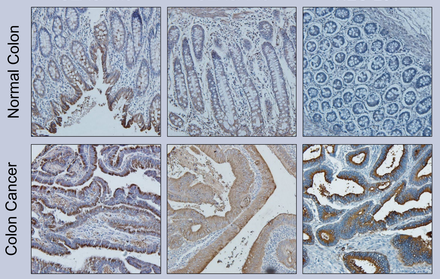
Tumor Cell Line Flow Cytometry



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



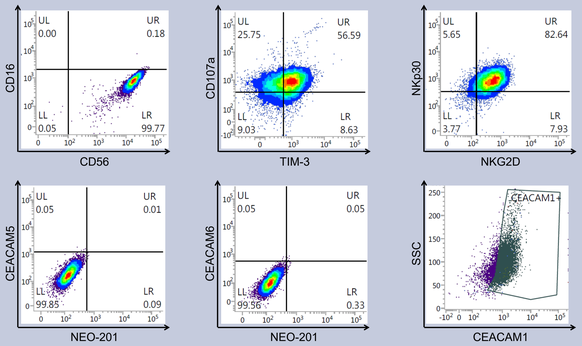
Quantification of Staining

Staining Pattern NEO-201	CEACAM5	CEACAM6	Normal Colon (Cases, %)	Colon Cancer (Cases, %)
-	-	-	**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.

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

NK-92 Cell Line Phenotype Analysis Flow Cytometry



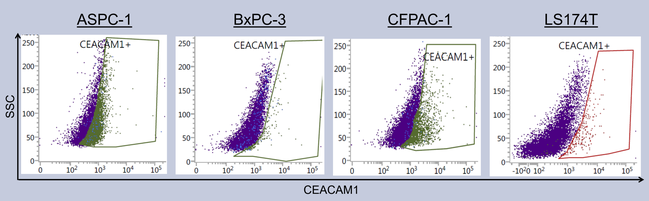
NK-92 cells are an immortalized IL-2-dependent human natural killer cell line that lacks ADCC activity.

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

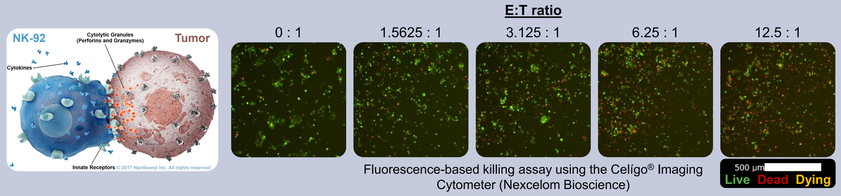


CELL LINE	% POS	MFI
NK-92	**82.57**	**1,270**
ASPC-1	**61.15**	**707**
BxPC-3	2.46	N/A
CFPAC-1	18.67	1,938
LS174T	2.43	N/A

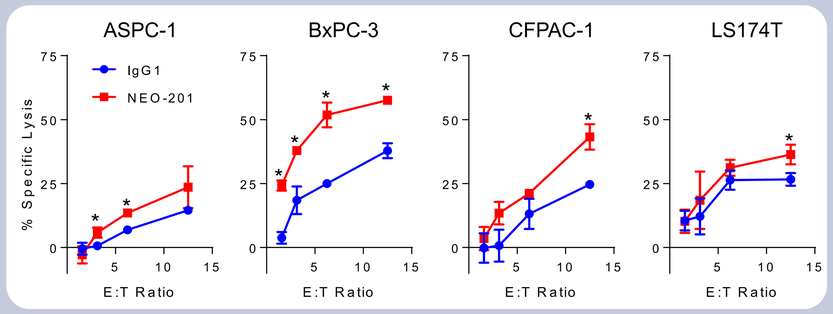
Only ASPC-1 cells highly express CEACAM1.

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

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



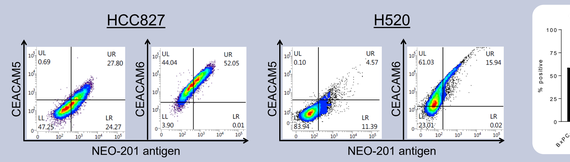
Fluorescence-based killing assay using the Celigo® Imaging Cytometer (Nexcelom Bioscience)



ASPC-1	BxPC-3	CFPAC-1	LS174T
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

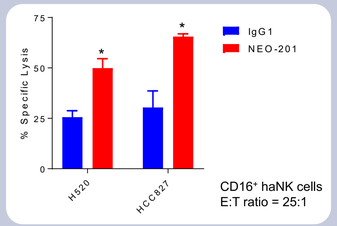
5. NEO-201-mediated enhancement of NK-92 killing depends on tumor cell CEACAM5 variant expression

Tumor Cell Line Flow Cytometry



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

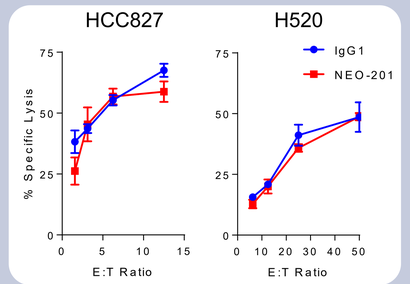
haNK 4hr ADCC Assay



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.

Additional information about NEO-201 can be found in our recently published manuscript:
Fantini & David *et al.* Preclinical characterization of a novel monoclonal antibody NEO-201 for the treatment of human carcinomas. *Frontiers in Immunology.* 2018 Jan 4;8:1899.

Companion abstract #619, Poster Section 27, Poster Board 13:
Fantini *et al.* ALT-803 enhances antibody-dependent cell-mediated cytotoxicity (ADCC) mediated by NEO-201 against human carcinoma cells.