PRECISION BIOLOGICS

The Discovery and Development of Novel Monoclonal Antibody, NEO-201 Targeting a Novel Neoantigen

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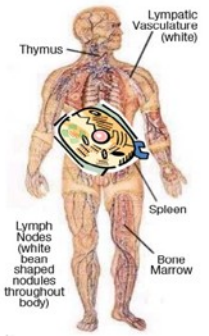
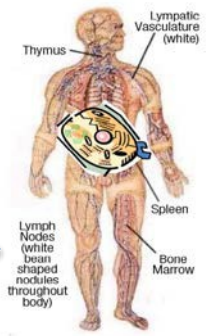
Only Human Derived & Human Tested Neo-antigen Platform to Create Novel Therapeutics



Patient-derived tumor samples

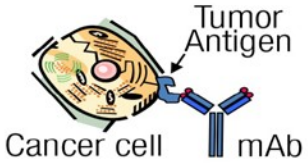
Preparation of cancer vaccines



Human screening to select active vaccine

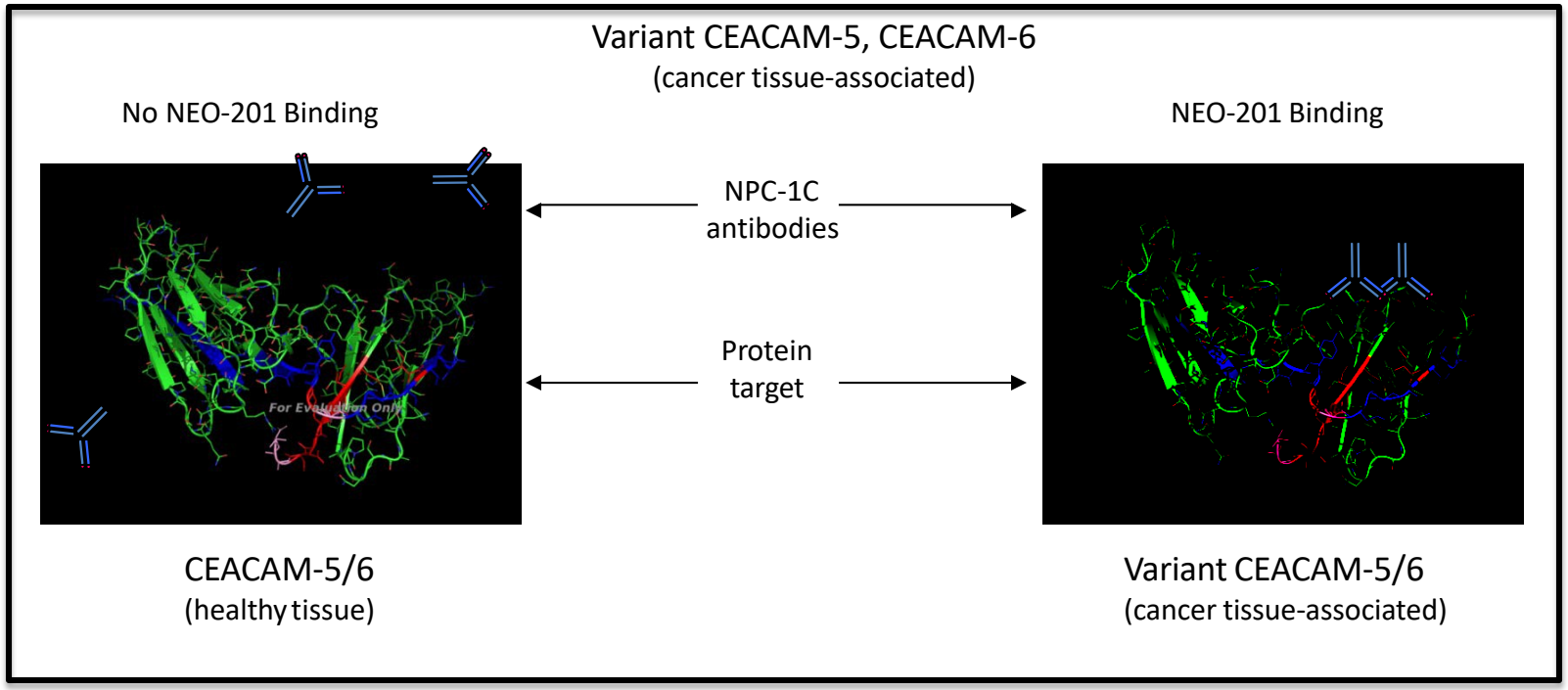
Hollinshead's clinical testing of anti-cancer vaccines

Production of anti-cancer-specific monoclonal antibodies



Testing and Development of mAbs

NEO-201 Target

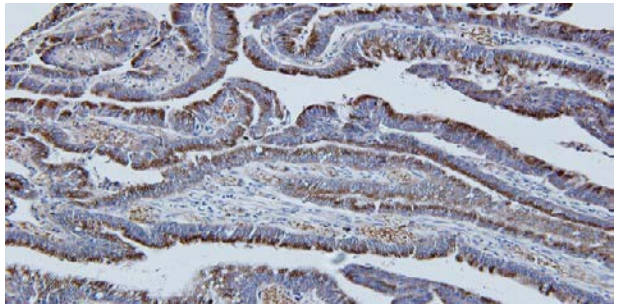


- NEO-201 recognizes tumor-specific variants of CEACAM-5 and CEACAM-6, members of the carcinoembryonic antigen (CEA) family of proteins. These proteins are expressed in normal epithelial tissues, and over-expressed in many solid tumor types (colon, pancreatic, breast, lung, ovarian)
- NEO-201 does not cross-react significantly with healthy tissues that express normal CEACAM-5 or CEACAM-6

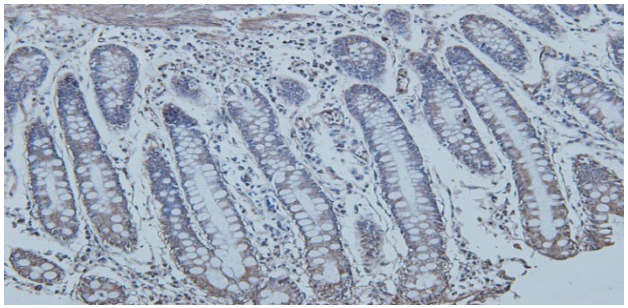
Comparison Binding Specificity of NEO-201 with Commercial CEACAM-5/6 Antibodies by IHC

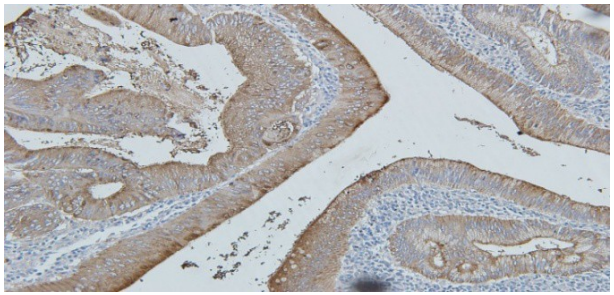
Colon cancer

Normal Colon

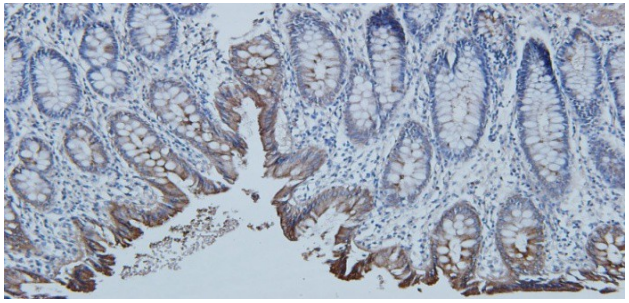


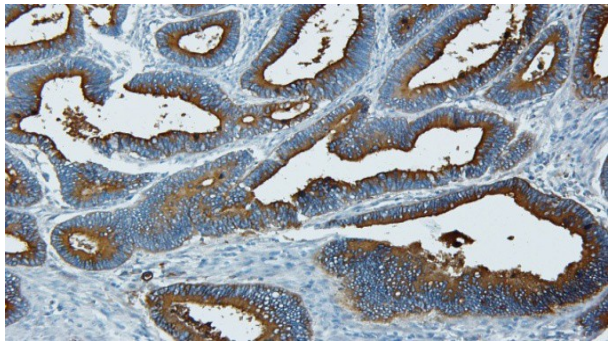
Anti-CEACAM-6
(9A6 from Cell Signal)



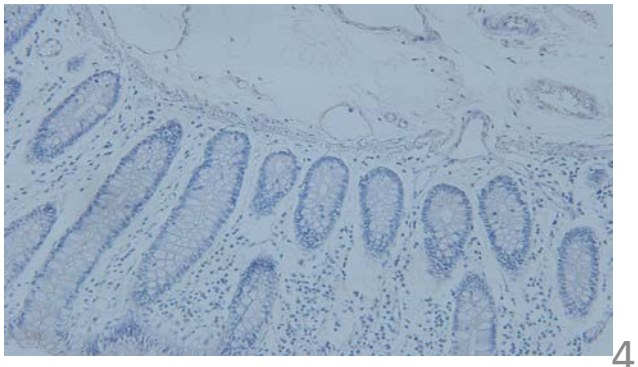


Anti-CEACAM-5
(CB30 from Abcam)



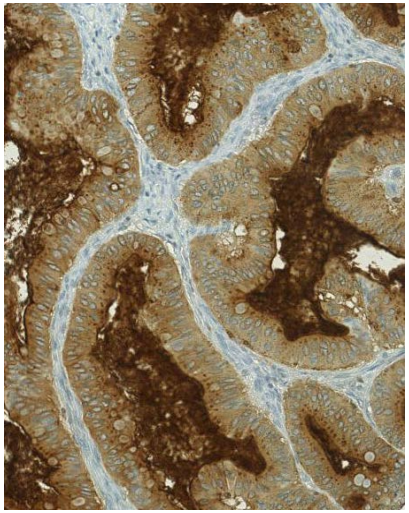


NEO-201

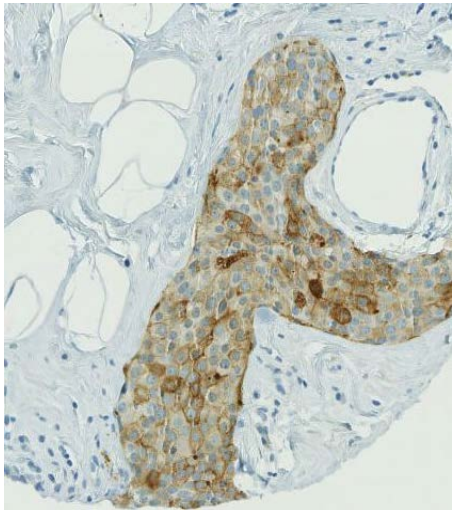


Collaborative Research Project – Precision Biologics and Christina Annunziata, MD, NCI

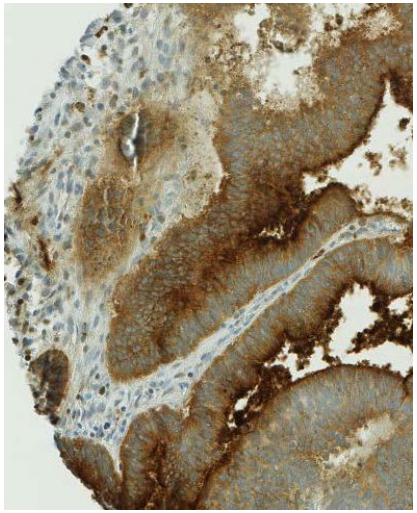
- Binds to tumor-associated antigen
- Minimal binding to healthy tissue



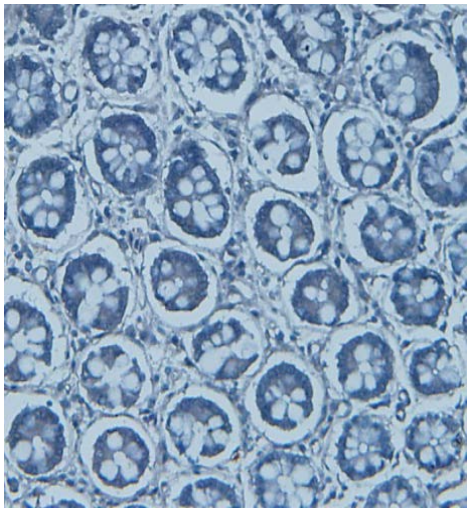
Ovarian Cancer



Breast Cancer

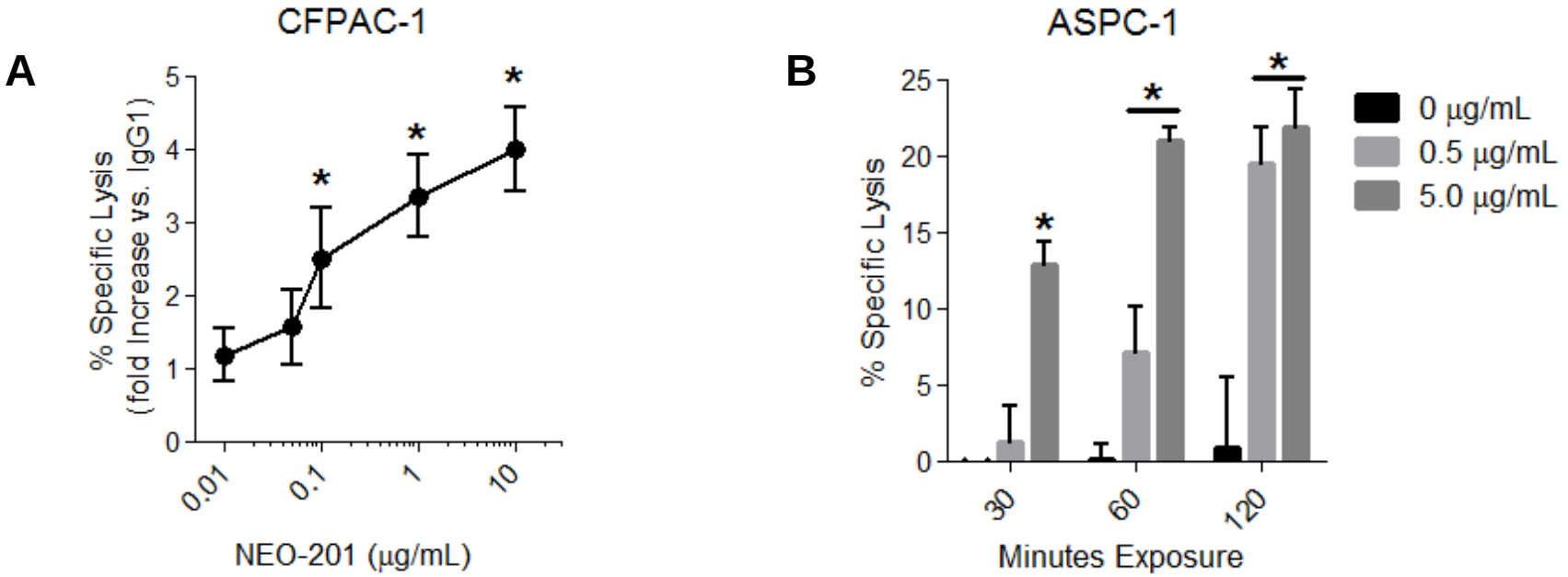


Colon Cancer



Normal colon

NEO-201 mediates ADCC and CDC against human tumor cells

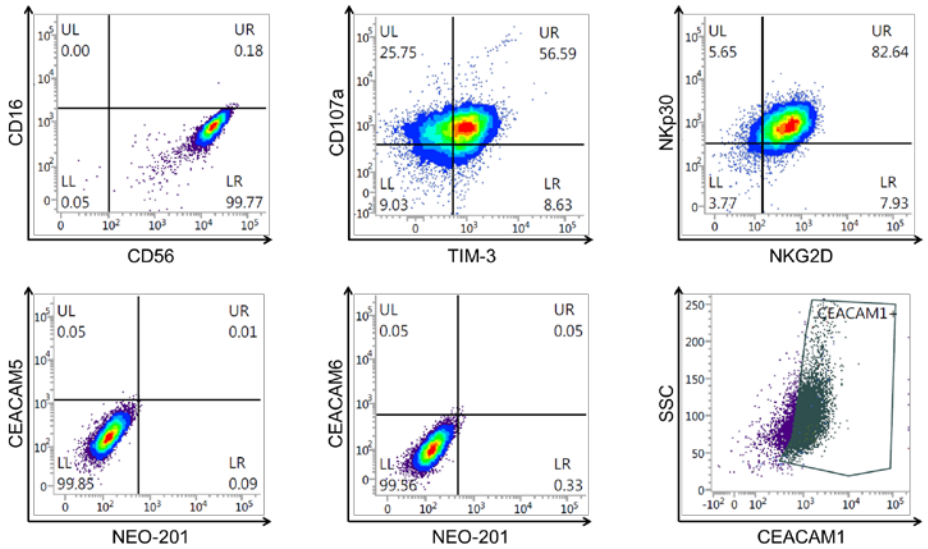


(A) ADCC assay using CFPAC-1 cells treated with increasing doses of NEO-201. NK cells isolated from a healthy donor were used as effector cells at an E:T ratio of 12.5:1. The graph depicts the fold increase in % specific lysis of NEO-201-treated tumor cells versus that of control cells treated with 10µg/mL human IgG1. (*, statistically significant ($p < 0.05$) by T-test.)

(B) CDC assay using ASPC-1 cells treated with rabbit complement (1:8 dilution) and the indicated doses of NEO-201 for the indicated durations. (*, statistically significant ($p < 0.05$) by T-test.)

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

NK-92 Cell Line Phenotype Analysis Flow Cytometry



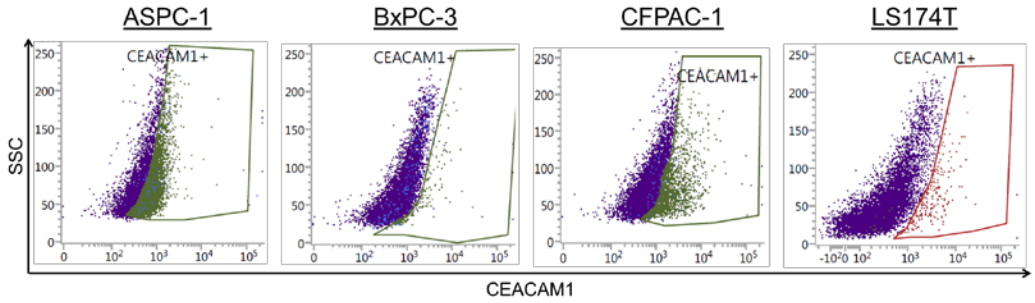
NK-92 cells are an immortalized IL-2-dependent human natural killer cell line that lacks ADCC activity.

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

CD56+	NK lineage marker
CD16neg	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

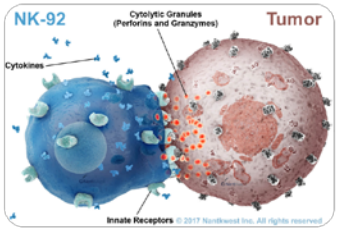


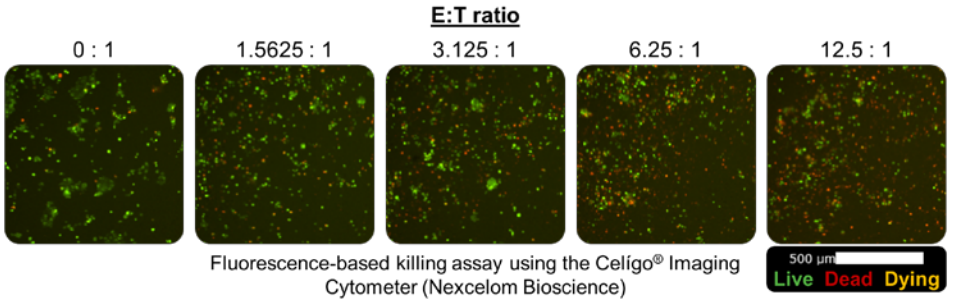
CELL LINE	% POS	MFI
NK-92	**82.57**	**1,270**
ASPC-1	**61.15**	**707**
BxPC-3	2.46	N/A
CFPAC-1	18.67	1,938
LS174T	2.43	N/A

Only ASPC-1 cells highly express CEACAM1.

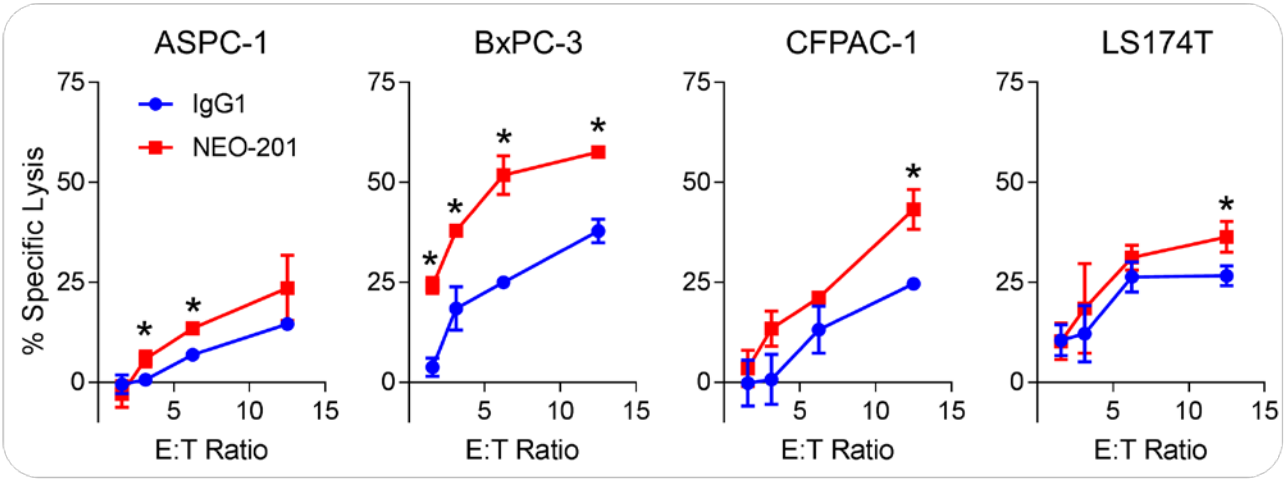
NEO-201 mAb enhances NK-92 cell cytotoxicity against CEACAM5+ / NEO-201+ tumor cells

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



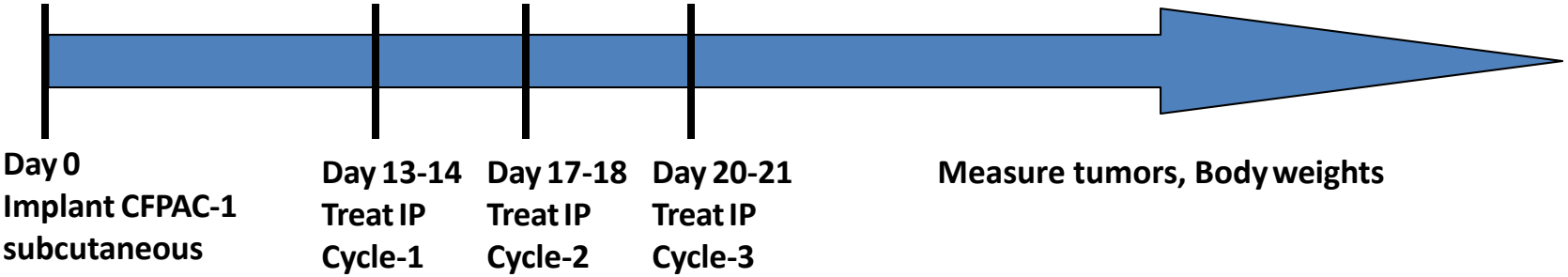


Fluorescence-based killing assay using the Celigo® Imaging Cytometer (Nexcelom Bioscience)



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

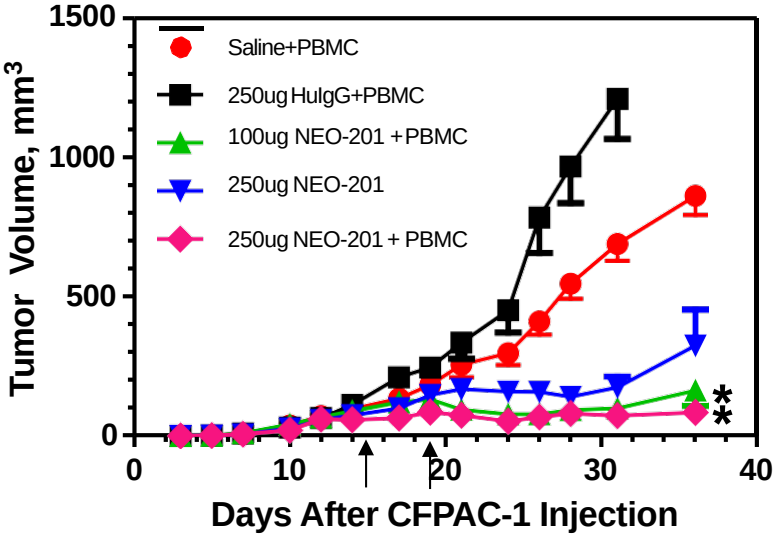
NEO-201(h16C3) Anti-tumor Efficacy in CFPAC-1 Tumor Xenograft Mouse Model



Group (n=10)	Antibody, dose	IL-2 Activated PBMC (NK cells)
1	Saline	~1x10^7
2	Human IgG, 250ug	~1x10^7
3	h16C3, 100ug	~1x10^7
4	h16C3, 250ug	No PBMC
5	h16C3, 250ug	~1x10^7

NEO-201 Anti-tumor Efficacy Results

NEO-201 Treatment of CFPAC-1 Tumor-Bearing Mice

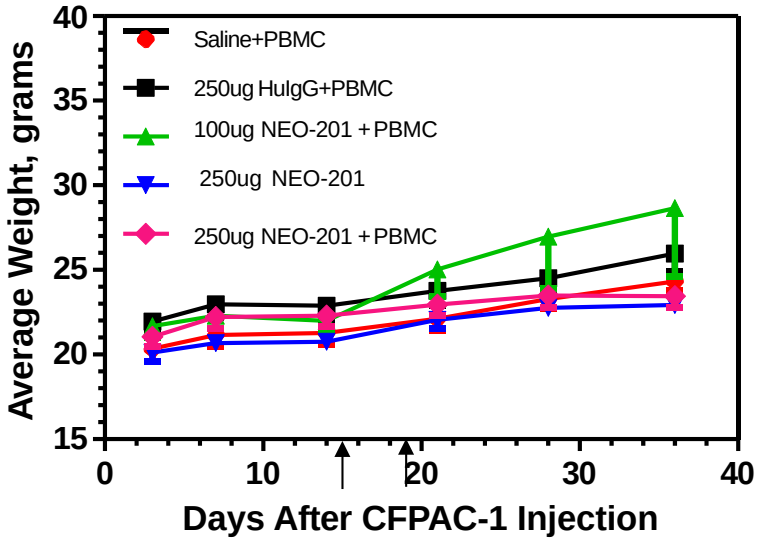


*, indicates $p<0.05$ vs. saline group

NEO-201 injected intraperitoneally on Days 13, 17, 20
PBMC injected intraperitoneally on Days 14, 18, 21

<u>On Day 36:</u>
100ug NEO-201+PBMC, 1/10 mice tumor-free
250ug NEO-201 +PBMC, 4/10 mice tumor-free

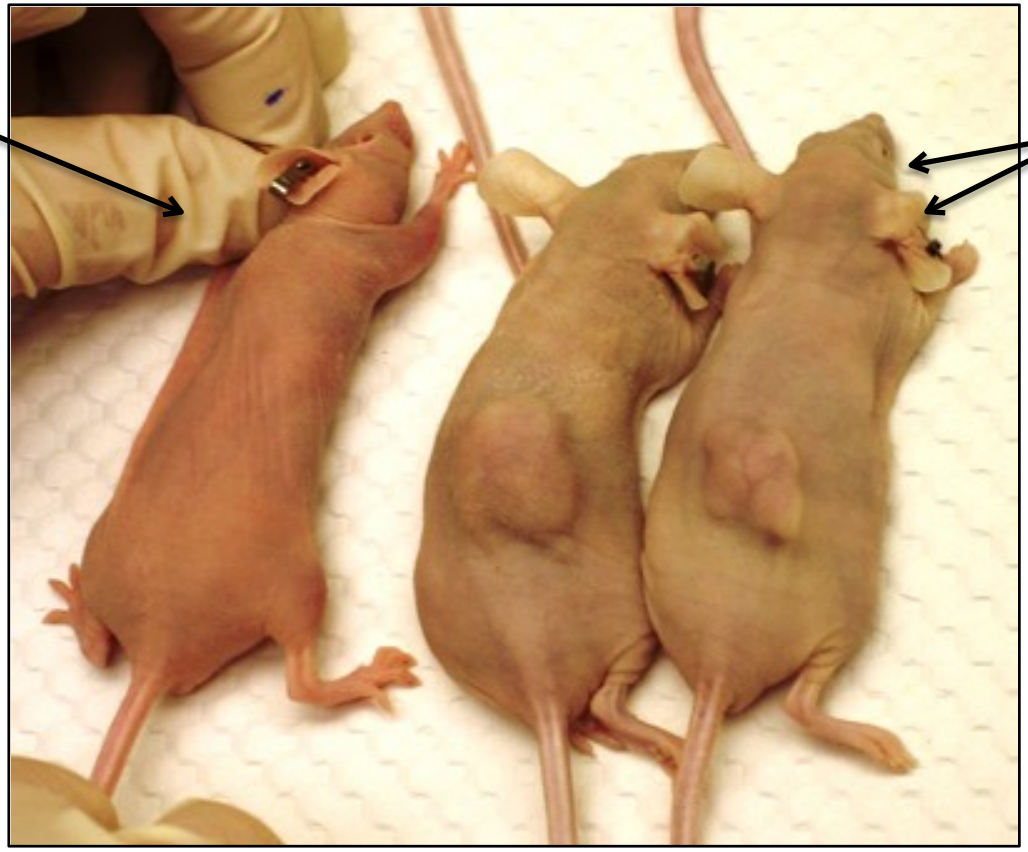
Body Weights, CFPAC-1 Tumor-Bearing Mice



NEO-201 injected intraperitoneally on Days 13,17, 20
PBMC injected intraperitoneally on Days 14, 18, 21

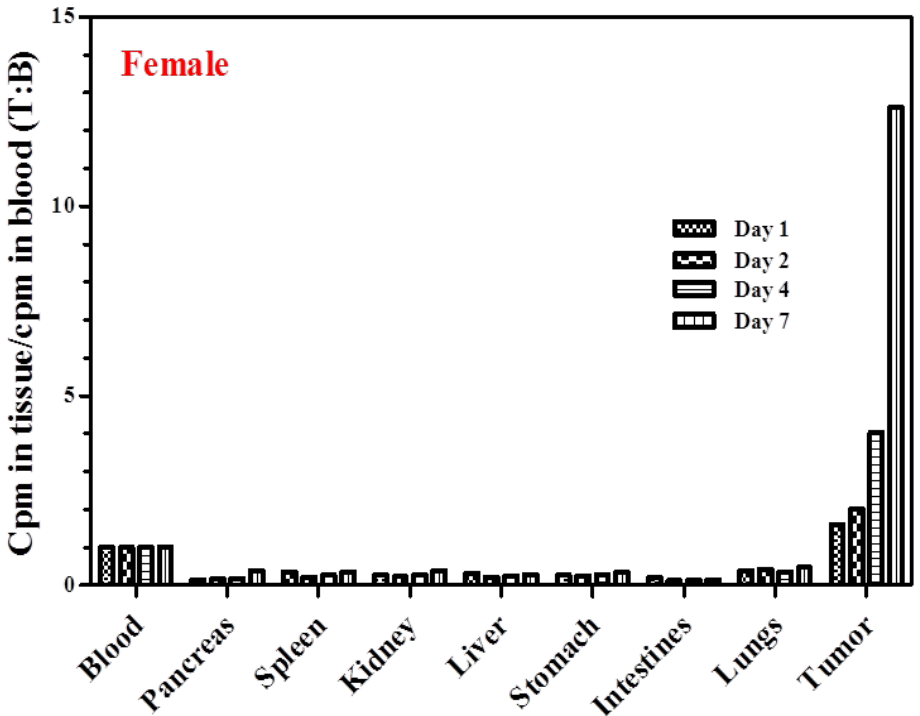
NEO-201 Anti-tumor Efficacy Results

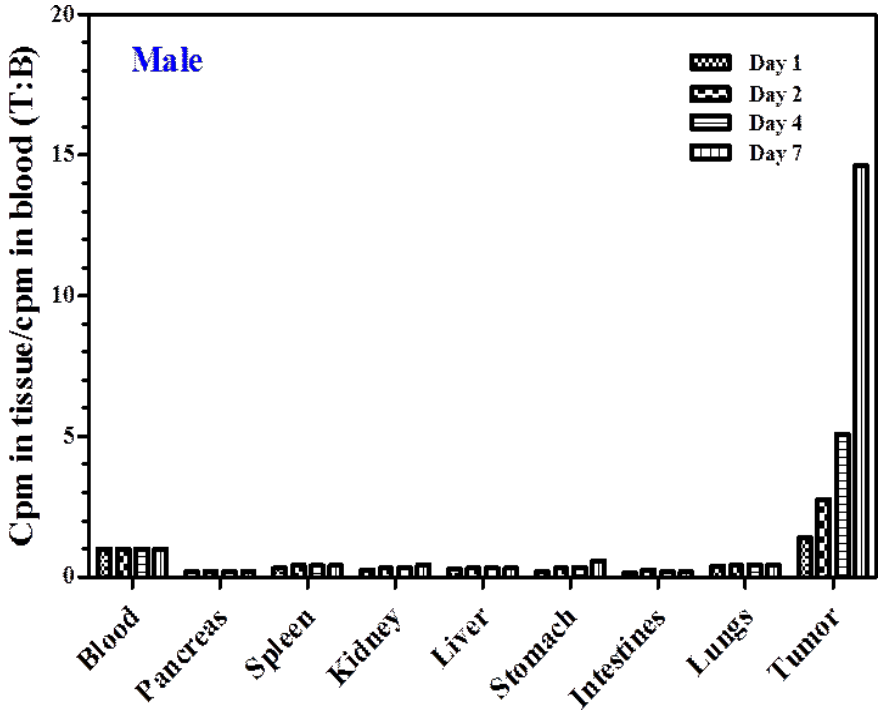
NEO-201 treated mouse



Control/Saline treated mice

NEO-201 Biodistribution Studies





Biodistribution studies show the distribution of NEO-201 in male and female mice with pre-established CFPAC-1 tumors. The mice were injected via tail vein with 20uCi of [^{125}I] labeled NEO-201 monoclonal antibody and necropsied after 1, 2, 4 and 7 days. Blood and tumors were collected. The following organs were removed: lungs, kidneys, liver, spleen, pancreas, intestines, and stomach. All tissues were weighed. Radioactivity in tissues was measured in a gamma counter, and data were calculated as cpm/mg tissue. The data shown above represent the normalization of tissue cpm relative to blood cpm.

NEO-201: FDA interactions and Drug Manufacturing

1. Pre-IND meeting held with FDA in 2017
   a. discussed relevant animal models
   b. pre-clinical studies required prior to IND filing
   c. GMP manufacturing of drug
   d. First in Human Clinical trial
2. GMP- manufacturing completed by Catalent Pharma Solutions
3. Drug Stability Completed and Drug Released for Clinical use

Nonclinical Testing of NEO-201: Selection of a Relevant Animal Species

1. As shown in the TCR studies, NEO-201 has cross reactivity with human granulocyte populations. Therefore, two studies were used to determine the most relevant animal population.

    a) IHC study using GI tissue from normal mice (both C57BL/6 and BALB/c), cynomolgus monkey, mini pig, cow and rat demonstrated staining of esophagus and colon of cynomolgus monkey and mice, but not the cow, rat or mini pig GI tissues.

    b) Flow cytometry of PBMCs from human, monkey and mouse was conducted to stain granulocytes with NEO-201. Only granulocytes from both monkey and human PBMCs stained similarly positive with NEO-201.

2. Hence the cynomolgus monkey was chosen as the relevant animal for nonclinical testing.

Nonclinical Testing of NEO-201: Single Dose Study

Study #1: Cynomolgus monkeys (8 male and 8 females)

- NEO-201 administered as one (1) intravenous dose
  - ✓ Group 1: 0 mg/kg NEO-201 IV (control group)
  - ✓ Group 2: 5 mg/kg NEO-201 IV (low dose)
  - ✓ Group 3: 20 mg/kg NEO-201 IV (moderate dose)
  - ✓ Group 4: 49 mg/kg NEO-201 IV (high dose)
- Pharmacokinetics (PK) samples were drawn pre-dose, 10 minutes, 1, 2, 4, 6, 24, 48, 72, 96, 168 and 336 hours

Nonclinical Testing of NEO-201: Single Dose Study

- Post NEO-201 infusion observations
  - ✓ No significant changes were observed in body weight, food consumption, urine, or laboratory testing.
  - ✓ Neutrophil count decreases were initially detected on Day 2 in Groups 2, 3, and 4, ranging from mild to moderate; all were transient with improvements by Day 8 and resolution by Day 15, although 2 males in Group 3 and one male and one female in Group 4 had persistently low neutrophil counts through Day 15.

CEACAM-6 is expressed on granulocytes, macrophages and monocytes, and hence this finding appears to reflect an on-target side-effect of NEO-201.

Nonclinical Testing of NEO-201: Single Dose Study

- NEO-201 PK Results
  - ✓ Mean TK results

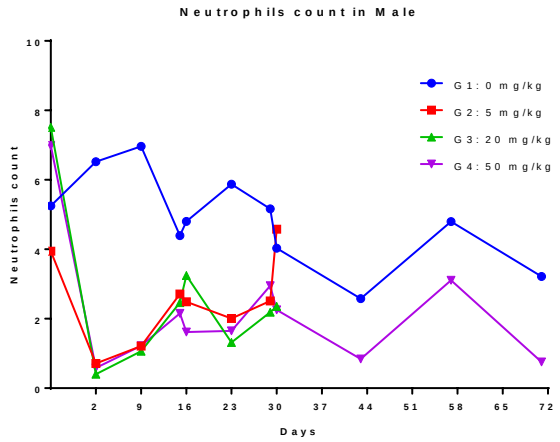
Group	HL (hr)	Tmax (hr)	Cmax (µg/mL)	Cmax/D (µg/mL/mg)	AUCinf (hr*µg/mL)	AUCinf/D (hr*µg/mL/mg)	CL (mL/hr)	Vz (mL)
2 (5 mg/kg)	46.2	0.584	138	11.4	8,220	680	1.54	103
3 (20 mg/kg)	167	0.167	579	11.2	70,100	1,360	0.746	179
4 (49 mg/kg)	170	0.167	1,470	11.8	157,000	1,260	0.830	191

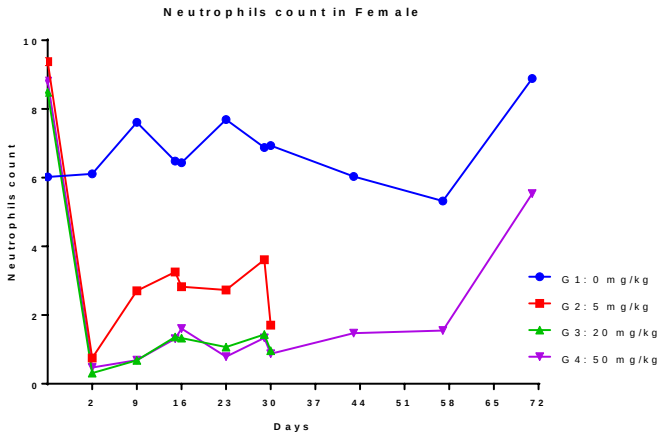
  - ✓ No difference between dose groups in Tmax (0.167 or 0.584 hour)
  - ✓ Peak (Cmax) exposure was dose proportional
  - ✓ Total (AUC) exposure was greater than dose proportional at the lowest doses and approximately proportional from 20 mg/kg to 49 mg/kg

Nonclinical Testing of NEO-201: Multi Dose Study

Study #2: 5-week GLP study with 6-week recovery in cynomolgus monkeys.

- Preliminary report showed similar findings to the observations made during the single dose study, i.e. no changes clinically, no significant changes in body weight, ECG, ophthalmology or urinalysis.
- Hematology laboratory changes were observed as depicted below:

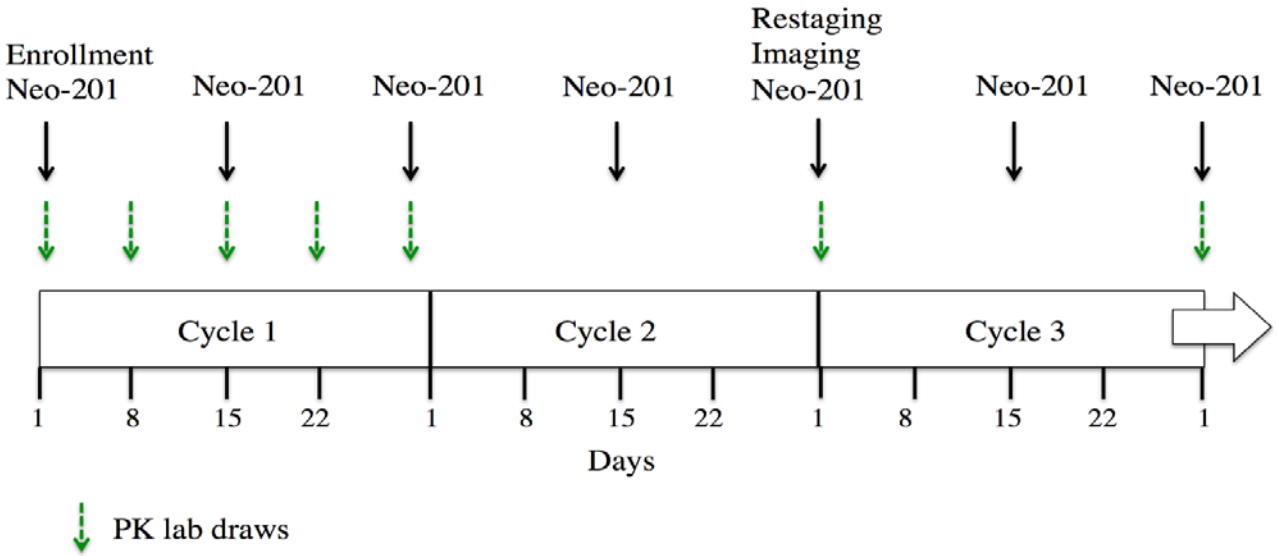




NEO-201 Planned Studies

- NEO-201- 1st in human studies will treat patients with colon ca, pancreatic ca, breast ca, NSCLC, and mucinous ovarian ca, who are no longer eligible for standard therapy .
- Phase I first in human study at NCI, open label, dose escalation study to determine safety and recommended phase 2 dose (RP2D) in patients with refractory cancers expressing NEO-201 antigen. RP2D will be explored in expansion cohorts in several targeted disease states at multiple centers.

Study Schema



NEO-201 Planned Studies

- Phase I first in human study at NCI:
  - Ongoing:

Dose Escalation Schedule		
Dose Cohorts	**Dose of IND Agent (mg/kg)**	**Number of Subjects planned for enrollment**
Level 1	1	3 - 6
Level 2	2	3 - 6
*Level 2.5***	*3*	*3-6*
Level 3	4	3 - 6
*Level 3.5***	*5*	*3-6*
Level 4*	6	3 - 6

*additional doses may be investigated if no DLTs or clinical activity is observed.
** dose de-escalation cohorts

  - Correlative studies to include:
    - Cellular immune monitoring assays (phenotype and functional [killing and suppression] assays with NK, Treg and MDSC; CD16 phenotype of NK/haNK cells);
    - Humoral immune monitoring assays (multi-plex cytokine and chemokine analysis; soluble factors such as MICA, arginase, soluble PD-1 and soluble PD-L1 and IDO, *in vivo* assay- HAHA analysis)

NEO-201 Planned Studies

- Development of biomarker
- Once the Recommended Phase 2 Dose (RP2D) is determined, the study will be expanded to include 4-5 cohorts of up to 35 subjects of different tumor types to gain further information on toxicity, PK, and preliminary clinical response data.
- Future Studies
  - Combinatorial approaches with NEO-201 to be determined based on analysis of the Phase 1 study and *in vitro* laboratory tests.

NEO-201 Summary

- Target is broadly expressed in multiple solid tumors.
- Exhibits both ADCC and CDC killing.
- Promising pre-clinical results with complete regression of tumor in animal models.
- GMP manufacturing completed.
- Pre-clinical testing showed that NEO-201 was well tolerated in cynomolgus monkeys even at high doses.
- Preclinical changes in hematologic laboratory values represents an on-target side effect that appears to be reversible, and supports infusion schedule of every two weeks.
- FDA authorized “Study may Proceed” letter on IND 7-2018
- NCI IRB-preliminary approval, pending final approval September 2018