- O-glycan array to test the binding activity of NEO-201 to 94 different O-glycan structures (figure 2A)

The anti-core 1 O-glycans targeting humanized monoclonal antibody (mAb) NEO-201 can also identify and kill immunosuppressive regulatory T (Tregs) cells and granulocytic myeloid-derived suppressor cells (gMDSCs) in human PBMCs

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Abstract #5654

Introduction

- NEO-201 is a humanized IgG1 mAb reactive against multiple human cancers but not against most normal epithelial tissues. NEO-201 binds to core 1 or extended core 1 O-glycans expressed by its target cells, including CD15+ granulocytes, solid malignant tumors and various human hematological neoplastic tumors.
- NEO-201 can mediate antitumor activity through multiple mechanisms of action such as antibody-dependent cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), and blockade of the CEACAM5/CEACAM1 immune checkpoint inhibitory pathway.
- NEO-201 does not bind to other immune subsets, such as B cells, NK cells, monocytes, or CD8+ T cells, and to the majority of CD4+ T cells.
- A previous study has demonstrated that about 4.6% of CD4+ T cells were positive for NEO-201 staining. Flow cytometry analysis revealed that NEO-201+/CD4+ T cells were also CD25+/CD127-/Foxp3+/CD15s+ in human PBMCs from healthy donors and cancer patients. NEO-201 can kill these Treg cells through CDC in vitro.
- Human gMDSCs likely originate from immature neutrophils and alternative activation of mature neutrophils.
- Since NEO-201 recognizes and kill human neutrophils through ADCC, the current study was designed to evaluate if NEO-201 can recognize and kill gMDSCs through ADCC.

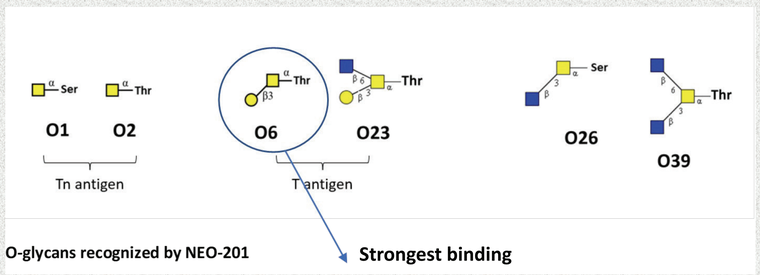
Experimental Design

- gMDSCs were generated from human neutrophils from 5 healthy donors. Neutrophils were isolated using the EasySepTM direct human neutrophil isolation kit. Isolated neutrophils were cultured in completed RPMI1640 medium supplemented with human GM-CSF (10ng/ml) and human IL-6 (10ng/ml) for 7 days and then profiled by flow cytometry.
- After 7 days of culture, generated gMDSCs were stained with NEO-201 and mAbs against human CD33, HLA-DR, CD15, CD14, CD66b . ADCC assay was performed using gMDSCs stained with both CD33 and HLA-DR as target cells. PBMCs from a healthy donor different from gMDSCs healthy donors were used as effectors cells with or without NEO-201 (10ug/ml) at the different E:T ratios.
- The ADCC activity of NEO-201 was evaluated comparing the percentage of CD33+/HLA-DRneg viable cells in gMDSCs incubated with medium alone with the percentage of CD33+/HLA-DRneg viable cells incubated with PBMCs alone and with PBMCs plus NEO-201.

Results

1. NEO-201 Binds to O-Glycans

O-Glycan array containing 94 different O-glycans to test the binding to NEO-201



01, 02, 06, 023, 026 and 039 O-glycans showed binding to NEO-201 in a dose-dependent manner.

The 06 is core 1 O-glycan and has the strongest binding to NEO-201

01 and 02 are Tn antigens.
023 is core 2, 026 is core 3 and 039 is core 4.

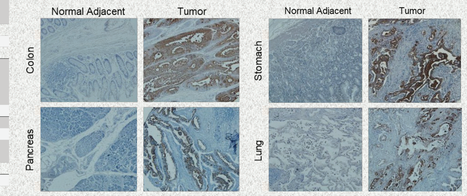
2. NEO-201 Binds to Several Human Carcinoma Cell Lines

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

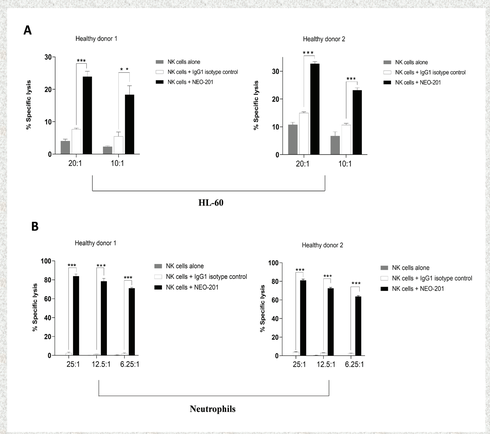
NEO-201 positive cell lines appear in bold text. NEO-201 positivity was defined as % positive >10%.

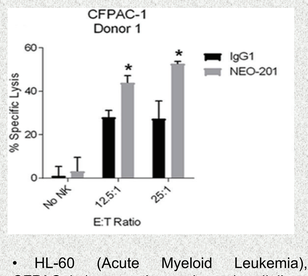
Immunohistochemistry



NEO-201 reacts against multiple human cancers but not against most normal epithelial tissues.

3. NEO-201 Mediates ADCC to Kill Target Cells Expressing Core 1 and Extended Core 1 O-Glycans

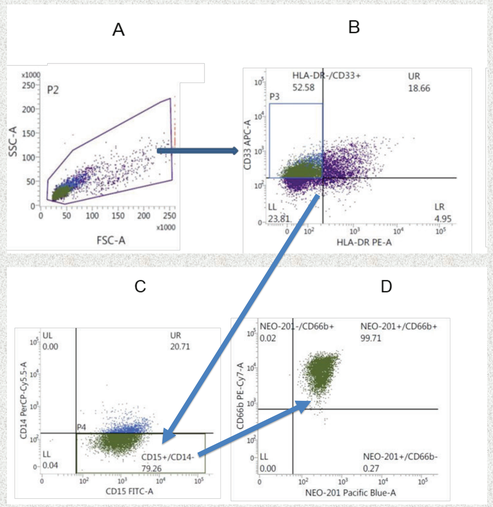




- HL-60 (Acute Myeloid Leukemia), CFPAC-1 (pancreatic carcinoma) cell lines and human neutrophils were used as target cells in the ADCC assay.
- Cells were incubated with 10µg/mL NEO-201 or human IgG1 (neg. control).
- NK cells from two healthy donors were used as effector cells at the indicated E:T ratios.

4. NEO-201 Targets Human gMDSCs

Phenotypic analysis of human gMDSCs as determined by flow cytometry

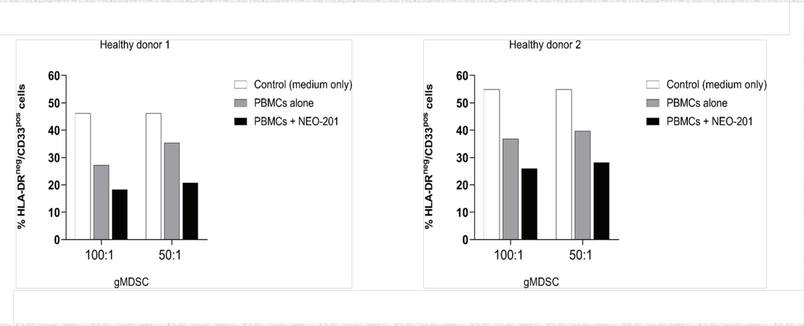


gMDSCs generated from Neutrophils

- Neutrophils were isolated using EasySep™ direct human neutrophil isolation kit.
- Isolated neutrophils were cultured in complete RPMI1640 supplemented with human GM-CSF (10ng/ml) and human IL-6 (10ng/ml).
- After 7 days, cells were then profiled by flow cytometry

5. NEO-201 Mediates ADCC to Kill gMDSCs

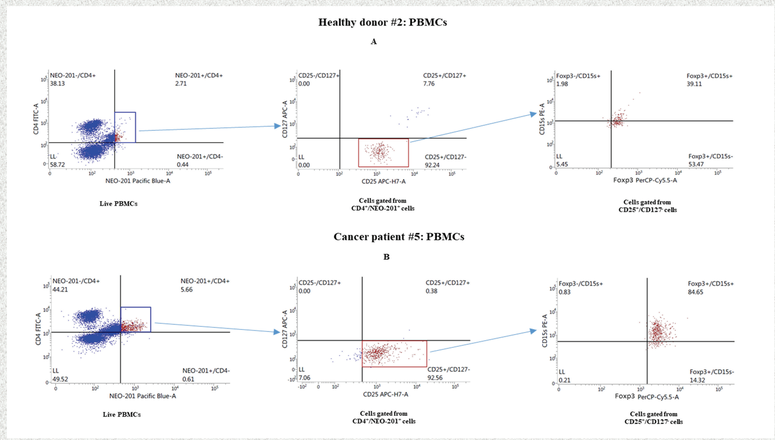
Flow cytometry based ADCC assay of 7-days culture of human gMDSCs



In gMDSCs incubated with PBMCs (E:T 100:1) plus NEO-201 we observed a **reduction of 33.01%** (18.29% vs 27.23%), **and 29.5%** (25.95% vs 36.83%) **of CD33^{pos}/HLA-DR^{neg} viable cells** compared to gMDSCs incubated with PBMCs alone (E:T 100:1) in healthy donor 1 and 2, respectively. Similar reduction of CD33^{pos}/HLA-DR^{neg} viable cells has been observed at E:T 50:1

6. NEO-201 Targets Tregs in Human PBMCs from Healthy Donors and Cancer Patients

Phenotypic analysis of human PBMCs as determined by flow cytometry



CD4$^+$/NEO-201$^+$ subset in human PBMCs from healthy donors and cancer patients has Tregs phenotype

Conclusion

This study demonstrated that NEO-201 can be used as a novel marker to identify both suppressive Treg cells and gMDSCs from human PBMCs. Furthermore, NEO-201 can kill gMDSCs through ADCC. Accumulation of Treg cells and gMDSCs in the TME is associated with low-rate response to checkpoint inhibitors in cancer patients.
Based on these data, we opened a phase II clinical trial combining NEO-201 with pembrolizumab for the treatment of solid tumors, with the hypothesis that NEO-201 may overcome resistance to checkpoint inhibitors therapies by depleting Tregs and gMDSCs.