Development of monoclonal antibodies targeting truncated O-glycans expressed specifically by cancer cells offers a novel strategy to enhance cancer immunotherapy efficacy

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Massimo Fantini, Anjum Zaki, Sharon A Mavroukakis, Philip M. Arlen, Kwong Y. Tsang·

Precision Biologics, Inc. Bethesda, MD 20814

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Introduction

Glycosylation is a vital post-translational modification of proteins and lipids in mammalian cells. One glycosylation pattern often disrupted during the process of carcinogenesis is O-glycosylation. The process O-glycosylation initiates by adding a single N-acetyl galactosamine (GalNAc) to either serine or threonine residue on selected proteins, resulting in the formation of O-glycans. O-glycans attached to amino acids on glycosylated proteins are involved in the activation of various physiological processes such as cell adhesion, cell-matrix interactions, cellular signaling, glycoprotein folding, cell differentiation, cell communication. Disruption of O-glycan formation leads to the expression of incomplete or truncated O-glycans on proteins of cancer cells from solid tumors (particularly in cancers of epithelial origin, including breast, ovarian, gastric, pancreatic, and colon cancers), as well as hematological malignancies. This alteration supports tumor progression and is often linked to poor prognosis.

Experimental Design

Targeting truncated O-glycans expressed specifically by cancer cells could be a promising strategy to enhance cancer immunotherapy efficacy, although it could be challenging.

We developed two monoclonal antibodies (mAbs), NEO-201 and NEO-102 that recognize truncated O-glycans expressed specifically on tumor tissues, sparing healthy tissues. Both mAbs were generated from the Hollinshead allogeneic colorectal cancer vaccine platform containing two immunogenic synergistic tumor associated antigens (TAAs) derived from pooled specimens from 79 patients with colon cancer. This cancer vaccine platform was used as immunogen to generate these two mAbs in mice. Our new mAb PB-223 was developed through immune engineering based on NEO-102.

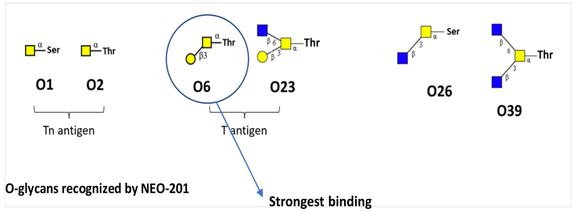
Results

1. Development and Characterization of NEO-201

NEO-201 is a humanized IgG1 mAb that binds to Core 1 and/or extended Core 1 O-glycans expressed by several human solid and blood tumors, as well as neutrophils, and mediates killing of cancer cells, neutrophils, regulatory T cells (Tregs) and granulocytic myeloid-derived suppressor cells (gMDSCs) via ADCC and CDC

NEO-201 Binds to Core 1 O-Glycans

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



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

Mechanisms of Action of NEO-201

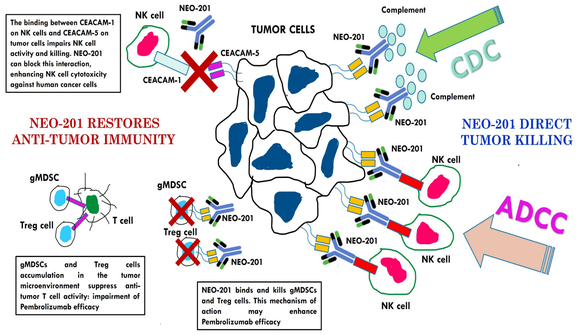
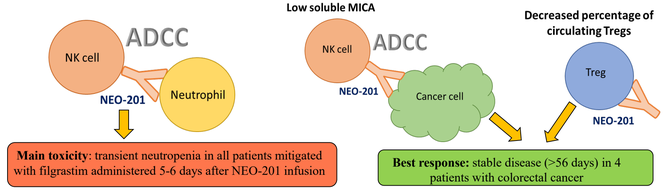


Figure modified from *Tsang et al. Cancers, 2022*

2. Safety and Efficacy of NEO-201 in Clinical Trials

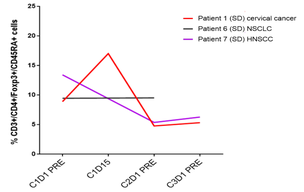
Phase 1 Clinical Trial: NEO-201 alone in patients with advanced solid tumors, that have not responded to standard treatments



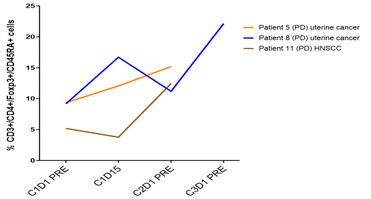
Cole et al. JECCR, 2023

Phase 2 Clinical Trial: NEO-201 + pembrolizumab in patients with tumors resistant to prior therapy with Immune checkpoint inhibitors (ICIs)

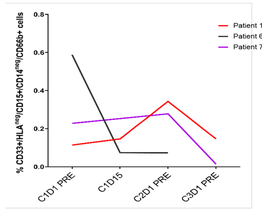
General downtrend of circulating naïve Tregs in patients with SD



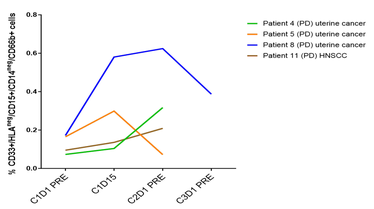
General upstream of circulating naïve Tregs in patients with PD



General downtrend of circulating gMDSCs in patients with SD



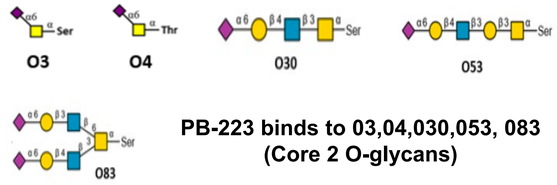
General uptrend of circulating gMDSCs in patients with PD



3. Development and Characterization of PB-223

PB-223 is a chimeric IgG1 mAb developed through the affinity maturation of mAb NEO-102

PB-223 Binds to Core 2 O-Glycans



PB-223 binds to 03,04,030,053, 083 (Core 2 O-glycans)

PB-223 Shows a Stronger Binding than NEO-102 to Cancer Cell Lines Expressing Core 2 O-glycans

Cell Line	Tumor Type	% NEO-102 Positive Cells (MFI)	% PB-223 Positive Cells (MFI)	Fold Increase
LoVo	Colorectal adenocarcinoma	0.90% (253)	1.01% (254)	1.12
SW-403	Colorectal adenocarcinoma	**22.21%** (62)	**51.23%** (102)	**2.31**
COLO-205	Colorectal adenocarcinoma	**10.30%** (48)	**41.05%** (78)	**3.98**
HCC1937	Triple negative breast cancer	8.39% (86)	**42.08%** (134)	**5.02**
OV-90	Ovarian adenocarcinoma	**24.66%** (392)	**43.12%** (469)	**1.75**
PC-3	Prostate adenocarcinoma	8.81% (97)	**13.23%** (107)	1.50

Bold and green: cancer cell lines with more than 1.5-fold increase in % of positive cells in flow cytometry after staining with PB-223 compared to NEO-102

NEO-102 showed good safety profile and encouraging survival results in Phase 2 clinical trial. Flow cytometry and IHC analysis showed **that PB-223 binds to a wider spectrum of human tumor types compared to NEO-102, while sparing normal tissues. PB-223 can also internalize into human cancer cell lines expressing core 2 O-glycans.**

Conclusions

NEO-201 and PB-223 are mAbs that specifically target O-glycans expressed on cancer cells while sparing healthy tissues. Clinical trial results indicate that NEO-201 demonstrates a manageable safety profile and promising efficacy by facilitating the destruction of both cancer cells and immunosuppressive cells expressing core 1 O-glycans. Additionally, NEO-201 and PB-223 serve as versatile platforms for developing next-generation therapeutics, including CAR-T/CAR-NK cells, T-cell/NK cell engagers, bispecific antibodies, and antibody-drug conjugates (ADCs).