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

Massimo Fantini1, David Justin1, Maria Pia Morelli3, Christina Annunziata2, Kwong Y Tsang1 and Philip M Arlen1.
1Precision Biologics, Inc., Rockville, MD, USA; 2Women’s Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

Abstract

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

Tumor Cell Line Flow Cytometry

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

ADCC assay

CDC assay

3. The NEO-201 antigen is a tumor-associated variant of CEACAM3 and CEACAM6

Tumor Cell Line Flow Cytometry

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

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

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

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

hNK 4hr ADCC Assay

NK-92 16hr Killing Assay

Background: NEO-201 is a humanized IgG1 monomeric antibody (mAb) that reacts to tumor-associated antigens (TAAs) derived from pooled allelogenic colon tumor tissue exsacinated by immunohistochemistry. NEO-201 exhibits both ADCC and complement-dependent cellular cytotoxicity (CDC) activity against human carcinoma cell in vitro, and counters 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 the 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 CEACAM6 on tumor cells inhibits activation signaling by NKGD2, which prevents NK cell cytolyis and permits tumor cells to evade NK killing.

This study was designed to 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 human 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. Flow cytometry experiments 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 CEACAM3.

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 CEACAM1 and NK cell CEACAM1 to reverse CEACAM1-dependent inhibition of NK cells. 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 CEACAMs.

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

Tumor Cell Line Flow Cytometry

NEO-201 is reactive against a broad range of in vitro cultured tumor cell lines. NEO-201 positive cell lines are depicted in green. NEO-201 positivity was defined as [positive %] > 95.

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

ADCC assay

CDC assay

ADCC activity using CFPRC-1 or ASPC-1 cells as targets. Cells were treated with 10µg/mL of NEO-201 or human IgG1 isotype control (isotype control). ADCC activity was determined after 18-24h incubation.

CDC assay using ASPC-1 cells treated with ratail complement (1/10 dilution) and the indicated doses of NEO-201 for the indicated durations.

3. The NEO-201 antigen is a tumor-associated variant of CEACAM3 and CEACAM6

Tumor Cell Line Flow Cytometry

This panel profiled tumor cell lines for a human-specific epitope recognized by NEO-201. NEO-201+ cells were monitoring in green. NEO-201 antigen expression was determined by FACS analysis.

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

This panel assessed the alterations in NK-92 expression upon treatment with NEO-201. NEO-201 killing efficiency was determined by the expression of the target of NK-92 killing and measured by flow cytometry.

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

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

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

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