Indirect mechanisms of action of a novel IgG1 monoclonal antibody, NEO-201, that enhance immune killing of tumor

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Introduction

NEO-201 is an IgG1 mAb targeting variants of CEACAM5 that demonstrates tumor sensitivity and specificity. Functional analysis revealed that NEO-201 is capable of engaging innate immune effector mechanisms including ADCC and CDC to directly kill tumor cells expressing its target. Previous studies demonstrated safety/tolerability in non-human primates, and an ongoing clinical trial at the NCI is currently exploring its dosing and safety. We have explored indirect mechanisms of its action that may enhance immune tumor killing. NEO-201 can block the interaction between CEACAM5 expressed on tumor cells and CEACAM1 expressed on NK cells to reverse CEACAM1-dependent inhibition of NK cytotoxicity. Phenotypic and functional analysis on human regulatory T cells (Tregs) in vitro also showed that NEO-201 can target and eliminate Tregs, suggesting that this tumor-targeting mAb may also mediate the down regulation of the Treg-mediated immunosuppression of anticancer immunity.

Experimental Design

Flow cytometry analysis and CDC assays were performed to evaluate the ability of NEO-201 to target and eliminate human Tregs in vitro. EasySep™ Human CD4+CD127+CD25− Regulatory T Cell Isolation kit and EasySep™ Human Biotin NEO-201™ Selection Kit (biotin-labeled NEO-201 mAb) were used to isolate Tregs from PBMCs from healthy donors. Phenotypic analysis was conducted by flow cytometry for the following markers: CD4, CD25, CD127, FoxP3, CD15s, CD45RA, NEO-201 antigen, CEACAM5 and CEACAM6. In addition, in vitro functional assays, using various human tumor cell lines as target cells and NK-92 cells (CEACAM1+CD16+) as effectors, were conducted to assess the ability of NEO-201 to enhance antitumor cytotoxicity of NK-92 cells by blocking the interaction between CEACAM5 on tumor cells and CEACAM1 on NK cells.

Results

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

2. NEO-201 binds to human regulatory T cells (Tregs)

Phenotypic analysis of isolated Tregs as determined by flow cytometry

3. NEO-201 mediates CDC activity against isolated CD4+CD15s−/CD25−NEO-201+ Tregs.

50% killing compared to untreated cells

Tumor Cells were isolated using the EasySep™ Human CD4+CD127−CD25− Regulatory T Cell Isolation kit

4. NEO-201 binds only to granulocytes in human hematopoietic cells

NEO-201 doesn’t react with other hematopoietic subsets (B cells, CD4+ T cells, CD8+ T cells, NK cells, monocytes)

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

Tumor Cell Line Flow Cytometry

NEO-201 and human IgG1 (negative control) were used at a concentration of 1 µg/mL. NK-92 cells were used as effector cells at the following E:T ratios: 1:5, 1:10, 2:5, 1:5, 5:1. Aldolase activity was determined using the following substrate concentrations: 0.25, 0.5, 1, 2 mg/mL. Wilcoxon rank sum test was used to determine statistical significance. NEC-92 vs NS-92 IgG1, p<0.05.