An anti-carcinoma monoclonal antibody (mAb) NEO-201 can also target and eliminate human immunosuppressive regulatory T cells (Tregs)

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Introduction

NEO-201 is an IgG1 mAb reactive against many different carcinomas, but not reactive against most normal tissues. Functional analysis revealed that NEO-201 is capable of engaging innate immune effector mechanisms to kill tumor cells, such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). In addition, 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.

Previous studies showed that NEO-201 attenuates growth of human tumor xenografts in mice and demonstrates safety/tolerability in non-human primates with a transient decrease in neutrophils being the only adverse effect observed. A clinical trial evaluating NEO-201 in adults with chemo-resistant solid tumors is ongoing at the NIH clinical Center.

Preclinical evaluation showed that NEO-201 reacts against human regulatory T cells (Tregs) and here we further investigated the phenotypic and functional effects of NEO-201 on human Tregs in vitro.

Experimental Design

Peripheral blood mononuclear cells (PBMCs) were collected from 5 healthy donors and used for phenotypic and functional analysis. EasySep™ Human Biotin NEO-201® Selection Kit (biotin-labeled NEO-201 mAb) were used to isolate Tregs from PBMCs. Phenotypic analysis was conducted by flow cytometry for the following markers: CD4, CD25, CD127, FoxP3, CD15s, CD45RA, CCR4, NEO-201 antigen, CEACAM5 and CEACAM6. The ability of NEO-201 to mediate killing of opsonized Tregs was evaluated using a CDC assay.

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%.

Positivity was determined using fluorescence minus one (FMO) controls. Positive cell lines were ranked according to their quantified expression level (% positive x 100), and then sorted into groups of low (<200), medium (200-1000), and high (>1000) expression.

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

Phenotypic analysis of isolated Tregs as determined by flow cytometry

EasySep™ Human CD4+CD127+CD25+ Regulatory T Cell Isolation kit (Healthy donor 1)

A: 86.27% of isolated cells are CD4+
B: 10.15% of CD4+ cells are CD25+/CD127+
C: 97.30% of CD4+CD25+/CD127+ cells are FOXP3+

D: 62.41% of FOXP3+ cells are NEO-201+CD15s
E: NEO-201+CD15s cells are CEACAM5+ and CEACAM5- negative

3. NEO-201+ Tregs are CCR4+

Phenotypic analysis of isolated Tregs as determined by flow cytometry

EasySep™ Human CD4+CD127+CD25+ Regulatory T Cell Isolation kit (Healthy donor 2)

A: 20.86% of CD4+ cells are CCR4+CD15s
B: 93.57% of CD3+CD127+ FoxP3+ cells are CCR4+/CD15s
C: 98.12% of CD25+/CD127- FoxP3+ cells are CCR4+/CD15s

D: 80% of CCR4+CD15s cells are NEO-201

4. The percentage of NEO-201+CD15s+ cells in the isolated Tregs ranges from 60%-80%

EasySep™ Human CD4+CD127+CD25+ Regulatory T Cell Isolation kit

DONOR % of NEO-201+CD15s+ cells in CD4+CD25+CD127+FOXP3+ cells
Healthy Donor 1 62.41
Healthy Donor 2 79.09
Healthy Donor 3 60.93
RANGE 60.93-79.09

EasySep™ Human Biotin NEO-201® Selection Kit

DONOR % of NEO-201+CD15s+ cells in CD4+CD25+CD127+FOXP3+ cells
Healthy Donor 4 50.76
Healthy Donor 5 99.12
RANGE 50.76-99.12

5. NEO-201 mediates CDC activity against isolated CD4+CD15s+NEO-201+ Tregs.

Healthy Tregs were isolated using the EasySep™ Human CD4+CD127+CD25+ Regulatory T Cell Isolation kit.

50 % killing compared to untreated cells.

Conclusion

This study demonstrates that NEO-201 reacts against human Tregs and can be used as a novel marker to identify and to purify Tregs. NEO-201 mAb could also eliminate NEO-201+ Tregs by CDC in vivo. This study demonstrates for the first time that this tumor-targeting mAb may also mediate through a novel mechanism down regulating Treg-mediated immunosuppression of anticancer immunity.