A therapeutic humanized anti-carcinoma monoclonal antibody (mAb) can also identify immunosuppressive regulatory T (Tregs) cells and down regulate Treg-mediated immunosuppression

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Introduction

NEO-201 is an IgG1 mAb reactive against many different human carcinomas expressing the NEO-201 antigen, but not against most normal epithelial tissues. NEO-201 can mediate antitumor activity against tumor cells through multiple mechanisms such as antibody-dependent cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), and blockade of the CEACAM5/CEACAM1 immune checkpoint inhibitory pathway. In addition to solid tumors, the NEO-201 target has also been found on specific subsets of human hematopoietic cells. Flow cytometry analysis has demonstrated that 98.9% of CD15$^+$ granulocytes and about 4.6% of CD4$^+$ T cells were positive for NEO-201 staining. No binding was observed with NEO-201 with respect to B cells, NK cells, monocytes, or CD8$^+$ T cells and a majority of CD4$^+$ T cells. A clinical trial evaluating NEO-201 in adults with chemo–resistant solid tumors is ongoing at the NIH clinical Center. This study was designed to characterize the subset of NEO-201$^+$ binding CD4$^+$ T cells and to evaluate the reactivity of NEO-201 to this subset of hematopoietic cells.

Experimental Design

Peripheral blood mononuclear cells (PBMCs) were collected from 10 healthy donors and 5 cancer patients enrolled in the open label, first-in-human, phase 1, dose escalation study started at NCI, NIH using NEO-201 in adults with solid tumors which have not responded to standard treatments. PBMCs were used for phenotypic and functional analysis.

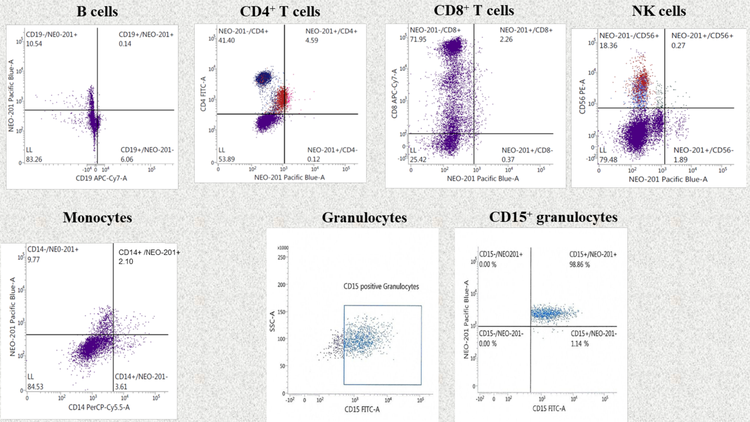
EasySep™ Human CD4$^+$CD127lowCD25$^+$ Regulatory T Cell Isolation kit and EasySep™ Human CD4$^+$ T Cell Isolation Kit were used to isolate Tregs and CD4$^+$ T cells from PBMCs. Phenotypic analysis of PBMCs was conducted by flow cytometry using the following markers: CD4, CD127, CD25, CD15s, Foxp3, CD39, CD73 and anti-NEO-201 mAb. The ability of NEO-201 to mediate killing of opsonized Tregs was evaluated using a flow cytometry-based CDC assay.

In the CDC assay, Treg cells, isolated from 3 healthy donors using the EasySep™ Human CD4$^+$CD127lowCD25$^+$ Regulatory T (Treg) Cell Isolation Kit, were used as target cells.

Results

1. NEO-201 targets CD15$^+$ granulocytes and a small population of CD4$^+$ T cells in human PBMCs from healthy donors

Hematopoietic Cells Flow Cytometry

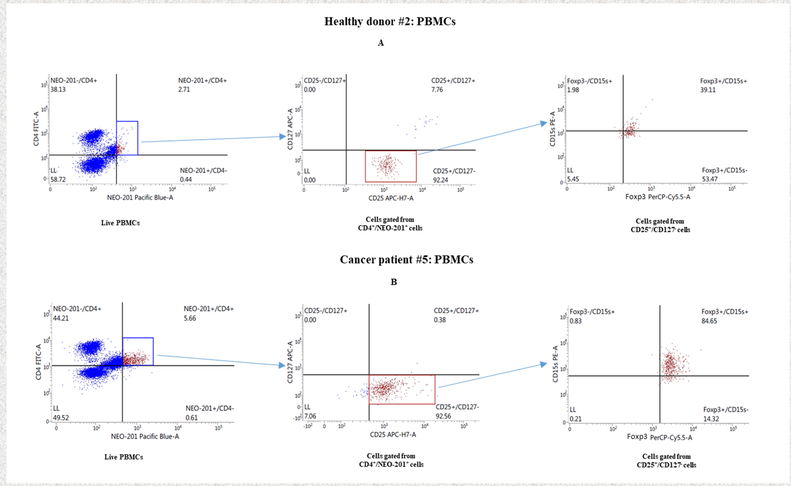


NEO-201 reacts with CD15$^+$ granulocytes and a small population of CD4$^+$ T cells

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

2. NEO-201 targets human immunosuppressive Tregs in human PBMCs from healthy donors and cancer patients

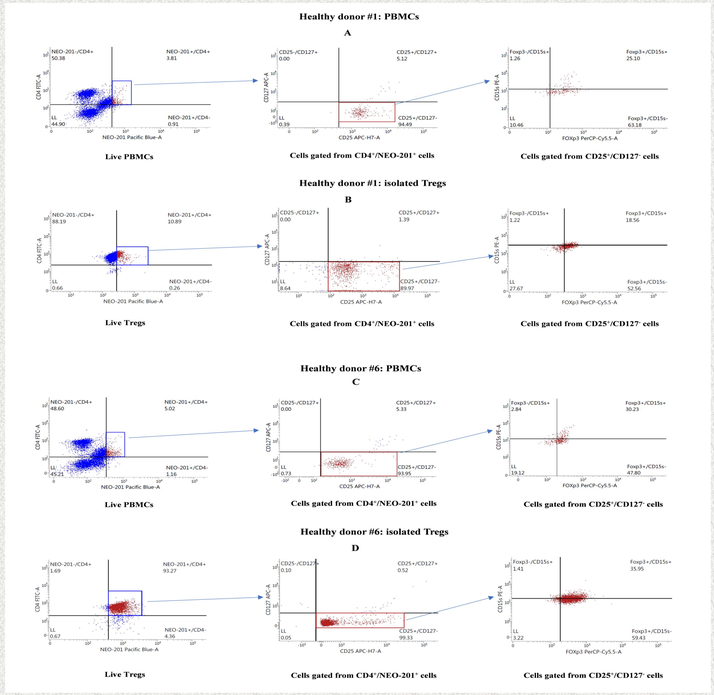
Phenotypic analysis of human PBMCs as determined by flow cytometry



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

Phenotypic analysis of isolated Tregs as determined by flow cytometry

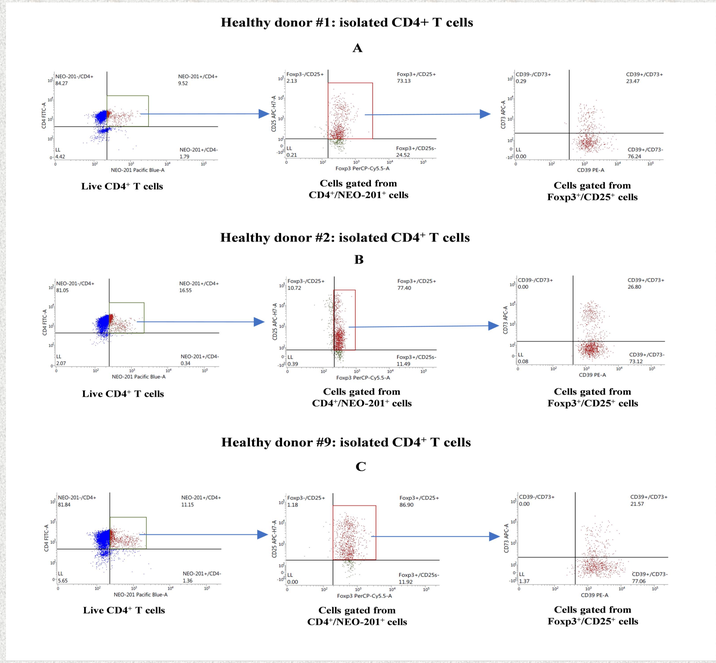
EasySep™ Human CD4$^+$CD127^{low}CD25$^+$ Regulatory T Cell Isolation kit



NEO-201 binds to isolated human CD4$^+$/CD25$^+$/CD127$^-$/Foxp3$^+$ Tregs

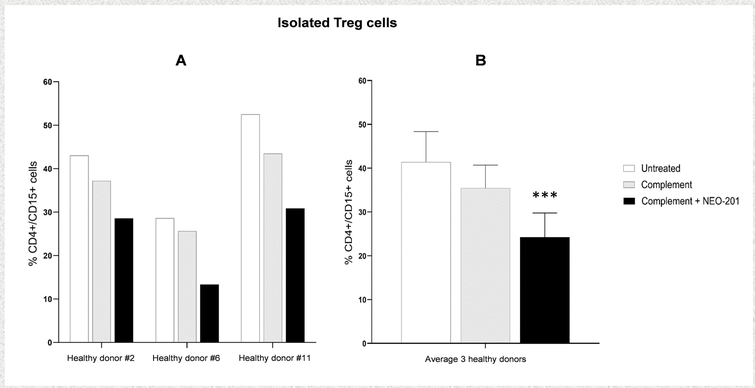
3. NEO-201 binds to CD39$^+$/CD73$^+$ and CD39$^+$/CD73$^-$ population in isolated CD4$^+$ T cells

EasySep™ Human CD4$^+$ T Cell Isolation kit



4. NEO-201 mediates CDC activity against isolated CD4$^+$/CD15s$^+$/NEO-201$^+$ Tregs.

Human Tregs were isolated using the EasySep™ Human CD4$^+$CD127^{low}CD25$^+$ Regulatory T Cell Isolation kit



Conclusion

This study demonstrated that the small subset of NEO-201$^+$CD4$^+$ T cells in human PBMCs are Treg cells and NEO-201 can be used as a novel marker to identify functionally suppressive Treg cells.

Furthermore, NEO-201 can kill Treg cells through CDC, presenting an opportunity for therapeutic intervention to increase anti-tumor immunity through the downregulation of the Treg-mediated immunosuppression of anticancer immunity.