# Identification of a Novel T Cell Co-Inhibitory Receptor and Potential Therapeutic Antibody Target in Oncology

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## Abstract And Introduction

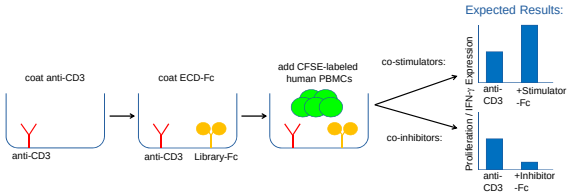
Antibody blockade of immune checkpoint regulators such as PD-1 and CTLA-4 has been shown to be an effective cancer treatment strategy; however, a large percentage of patients still do not respond to existing therapies. Discovery of additional immune checkpoints and development of antibody therapeutics against them are likely critical to address this unmet patient need. We generated a comprehensive library of essentially all human extracellular proteins and screened a subset of proteins in this library *in vitro* and *in vivo* for the ability to modulate immune responses or tumor growth. As a result of these screens, we identified a number of novel T cell co-inhibitors[1].

One such protein, referred to herein as Novel Co-Inhibitor 1 (or NCI1), was originally identified through its inhibitory activity on anti-CD3-stimulated human T cell proliferation. To confirm its activity as a T cell co-inhibitor, we demonstrated that the native protein expressed on an antigen-presenting cell line could inhibit antigen-stimulated CD8+ T cell activation. Furthermore, blocking antibodies against this protein relieved the inhibition. This inhibitory activity translated to a murine system, as the mouse ortholog and blocking antibodies behaved similarly in murine T cell activation assays. Overexpression of the protein in mouse syngeneic tumor models resulted in increased tumor growth, consistent with it inhibiting tumor-infiltrating T cells. Novel Co-Inhibitor 1 is expressed primarily on activated and regulatory T cells in humans and mice – an expression profile similar to those of PD-1 and CTLA-4. Additionally, it is expressed on higher percentages of tumor-infiltrating T cells than on circulating T cells in mice. We are currently evaluating the anti-tumor activity of blocking antibodies in mouse tumor models, either alone or in combination with other checkpoint blocking antibodies. Taken together, we believe that these data demonstrate that this newly discovered protein may act as a checkpoint regulator in tumors and that blocking antibodies against it have potential as a novel cancer immunotherapeutic.

1. Sallee, Lee, Rankin, Halenbeck, Brace, Williams, Wong, Kavanaugh: Discovery of Novel Immune Checkpoint Regulators in a Comprehensive Library of Human Extracellular Proteins. SITC 2014 poster.

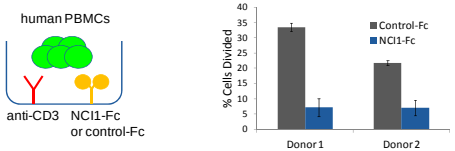
## Methods: Designing A Screen For Novel T Cell Regulators

- The extracellular domains (ECDs) of human proteins were expressed as IgG1 Fc fusions and purified by protein A chromatography, using our high-throughput expression platform
- We developed an assay to screen for the ability of library proteins to modulate human T cell activation
96-well plates were first coated with anti-CD3 (a T cell activator) and then the wells were coated with individual library proteins. Human CFSE-labeled PBMCs were incubated in the coated plates and then T cell proliferation and IFN-γ expression were measured.

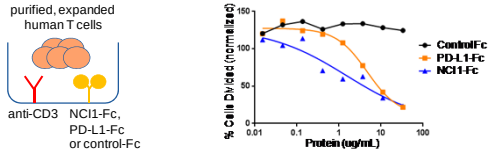



## Novel Co-Inhibitor 1 (NCI1) Was Identified As A T Cell Inhibitor

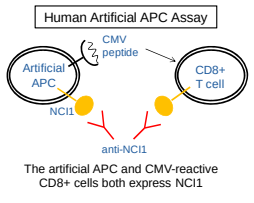
- Screen data showed that NCI1 inhibited proliferation of T cells within PBMCs:

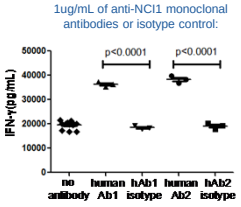



- NCI1 also inhibited the proliferation of purified, pre-activated CD4 and CD8 T cells:



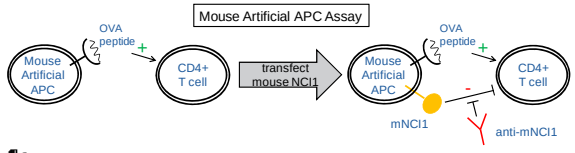

## Native, Transmembrane Novel Co-Inhibitor 1 Inhibits T Cells And This Can Be Reversed By Anti-NCI1 Antibodies

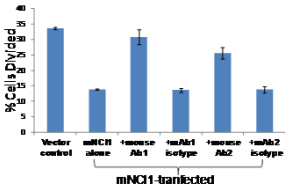


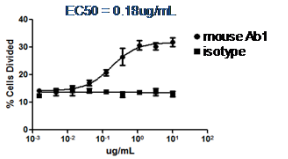

- This stimulation is also seen with anti-NCI1 Fabs, so it is not dependent on the Fc domain or crosslinking

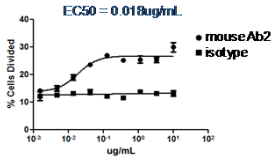
## The Mouse Ortholog Of Novel Co-Inhibitor 1 Also Inhibits T Cell Activation And Can Be Blocked By Antibodies



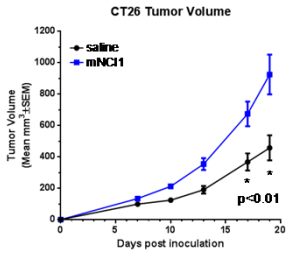

- Mouse NCI1 (mNCI1) inhibits OVA-stimulated CD4 T cell proliferation
- Anti-mNCI1 antibodies neutralize this inhibition
- Mouse Ab2 (mAb2) blocks 10-fold more potently than mouse Ab1 (mAb1)

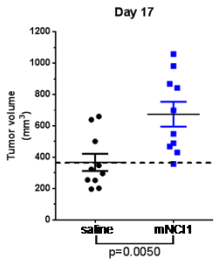



## Mouse Novel Co-Inhibitor 1 Accelerates Tumor Growth In The CT26 Mouse Colorectal Tumor Model

- Mouse NCI1 – Human IgG2 Fc was over-expressed in the circulation of mice using a proprietary systemic expression technology and CT26 tumors were inoculated subcutaneously one week later

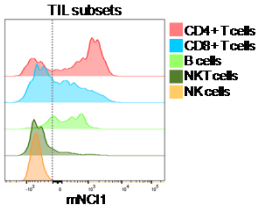


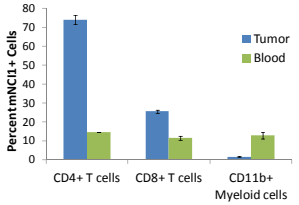




- Significantly increased growth was demonstrated relative to saline control
- This is consistent with mNCI1 binding to and inhibiting tumor-infiltrating T cells
- We also saw increased tumor growth with mNCI1 in the E.G7-OVA lymphoma model

## Mouse Novel Co-Inhibitor 1 Is Highly Expressed On Tumor-Infiltrating T Cells

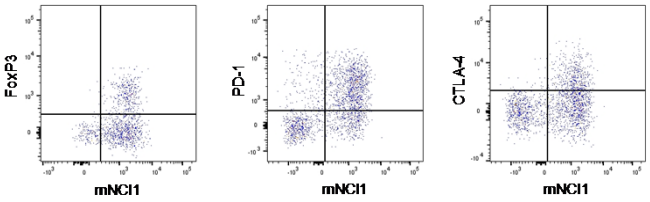
Flow cytometry staining of tumor-infiltrating leukocytes (TILs) in the CT26 model




- mNCI1 protein has selectively enriched expression on T cells in mouse tumors
- Some circulating myeloid cells express mNCI1, but not in the tumor
- mNCI1 is not expressed on the tumor cells themselves
- Similar expression profile seen in MC38 and 4T1 tumor models

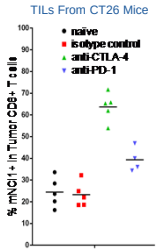
## Mouse Novel Co-Inhibitor 1 Co-Expresses With FoxP3, PD-1 And CTLA-4 On Tumor CD4+ T Cells




- Of the FOXP3+ CD4+ T cells in the tumor, 99% also express mNCI1
- Of the PD-1+ CD4+ cells in the tumor, 88% also express mNCI1
- Of the CTLA-4+ CD4+ cells in the tumor, 94% also express mNCI1

## Targeting Novel Co-Inhibitor 1 In Mouse Tumor Models

- We are currently determining whether blocking mNCI1 in mouse tumor models can reduce tumor growth
- We tested either mouse Ab1 or Ab2 as single agent therapies in two different syngeneic tumor models
- We saw clear evidence of engagement of mNCI1 in the tumors, but neither antibody had a significant effect on tumor growth as a single agent
- We are now testing anti-mNCI1 in combination with anti-PD-1 or anti-CTLA-4 in multiple tumor models
- We have observed that CT26 tumor-bearing mice treated with anti-PD-1 or anti-CTLA-4 as a single agent have increased expression of mNCI1 in their tumor CD8+ T cells – possibly as a compensatory mechanism




## Conclusions And Future Directions

- The human Novel Co-Inhibitor 1 – IgG1 Fc fusion protein inhibits purified T cells. Human and mouse NCI1 also inhibit in artificial APC assays as native, transmembrane proteins
- The mouse Novel Co-Inhibitor 1 – human IgG2 Fc fusion protein accelerates tumor growth in two syngeneic tumor models, consistent with T cell inhibition
- The expression of Novel Co-Inhibitor 1 is specifically enriched in mouse tumor T cells. It also co-expresses with PD-1, CTLA-4 and FoxP3 in tumor CD4+ T cells
- The mechanism of action by which Novel Co-Inhibitor 1 is working is not defined yet and we are attempting to characterize it
- We are currently evaluating the efficacy of Novel Co-Inhibitor 1 blocking antibodies in mouse tumor models
- These results demonstrate the ability of Five Prime's discovery platform to identify novel immune regulatory proteins and to evaluate their potential as immuno-oncology therapeutic targets