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 to the ability to modulate immune responses or tumor growth. As a result of these screen, we identified a number of novel T cell co-inhibitors.

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 its 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 that of PD-1 and CTLA-4. Additionally, it is expressed in 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.

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 OFSE-decorated PBMCs were incubated in the coated plates and then T cell proliferation and (IFN) 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:

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

Conclusions And Future Directions
- The human Novel Co-Inhibitor 1 – IgG2 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—possibly as a compensatory mechanism.
- 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.