Identification Of Novel T Cell Co-inhibitory And Co-stimulatory Receptors From Screening A Comprehensive Library Of Extracellular Proteins

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Introduction

We have developed multiple sophisticated in vitro, in vivo, and biophysical assay systems for screening our comprehensive library of human extracellular proteins in order to identify novel therapeutics that can reprogram immune cells in the tumor microenvironment. In vitro screens of primary human effector and regulatory T cells identified novel proteins that can modulate T cell activation and suppression. We also screened subsets of our library in four distinct syngeneic mouse tumor models (either as single agents, or in combination with checkpoint blockade or vaccines) and identified targets that can modulate an anti-tumor immune response in vivo. In addition, we performed a combinatorial biophysical screen for protein-protein interactions within a set of ~700 predicted immune-related transmembrane proteins and identified novel receptor-ligand interaction networks. Using this multi-pronged screening approach, we have confirmed activities of well-known immunomodulatory proteins including GITRL and CD80. Moreover, we have identified novel immune modulators that have activities across multiple in vitro and in vivo assays, as well as novel interaction partners for these proteins that help us elucidate their mechanisms of action. One such example is OLR1, which we found to be a novel co-stimulator of effector T cells, potentially through a novel interaction with TM9SF1. Additionally, we identified previously unappreciated mechanisms for proteins with known immunomodulatory properties. One such example is soluble DLL4, which we found to specifically stimulate regulatory T cells and to modulate adaptive immune responses in a tumor model in vivo. Taken together, we have developed robust in vitro and in vivo platforms that allow us to discover new immune-modulatory therapeutics that we believe will help address the needs of cancer patients that fail to respond to current immunomodulatory therapies.

Regulatory T Cell Screen

Effector T Cell Screen

- Engineered K562 artificial antigen presenting cell.
- Cell-surface anti-CD3 fragment to stimulate T cells.
- Non-signaling CD64 Fc receptor to capture library proteins.
- Human pan T cells pre-activated, then rested.
- Incubated with FivePrime library of extracellular domain (ECD) - Fc fusion proteins.
- Identified known and novel T cell co-stimulators and co-inhibitors.

In Vivo Screens In Multiple Syngeneic Tumor Models

- Screened a library subset in 4 syngeneic tumor models with unique immune cell infiltrate profiles: CT26, MC38, 4T1 in combination with anti-PO-1, and TC-1 in combination with peptide vaccine

Summary And Next Steps

- We are taking multiple approaches to identifying novel immune modulatory pathways:
  - In vitro screens of Effector T cells and Tregs
  - Biophysical screen for interactions among immune receptors
  - In vivo screens in syngeneic mouse tumor models

- Each of these approaches have identified activities and interactions of many known immune modulators
- In addition we have identified:
  - Novel immune regulatory pathways
  - For example: OLR1- TM9SF1 from the Treg and Immunomodulatory screens
  - Unexpected mechanisms from known immune modulators
  - For example: DLL4 from the Treg and in vivo screens

- Combined data from multiple screening approaches we have prioritized a series of new targets for validation as potential novel immuno-oncology therapeutics

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