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

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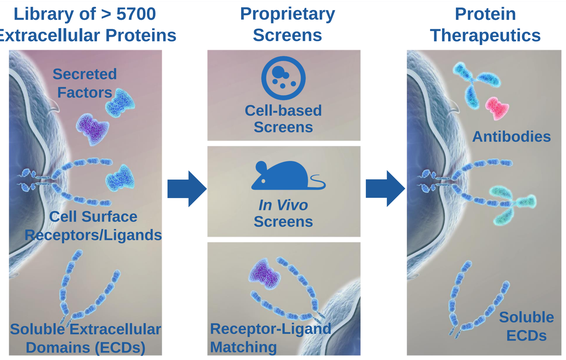
Introduction

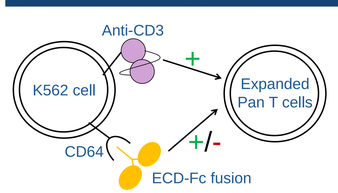
We have developed multiple sophisticated *in vitro*, *in vivo,* and biophysical assay systems for screening our comprehensive library of human extracellular proteins in order to identify novel therapeutics that can reprogram immune cells in the tumor microenvironment. *In vitro* screens of primary human 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 vaccine) 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 immuno-therapeutic targets that we believe will help address the needs of cancer patients that fail to respond to current immunomodulatory therapies.

FivePrime's Unique Platform: Screening A Library Of The Extracellular Proteome To Identify Novel Targets And Therapeutics

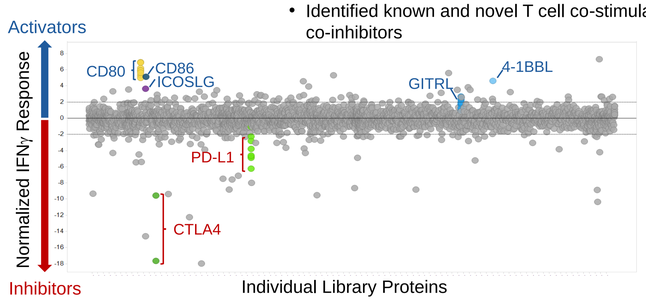


- Secreted proteins and extracellular domains of membrane bound proteins are expressed in a human cell system
- The proteins are screened for functional activity in cell based or biophysical binding studies
- DNA encoding mouse secreted proteins is delivered directly into mouse disease models

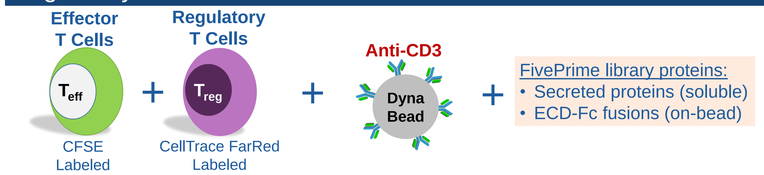
Effector T Cell Screen



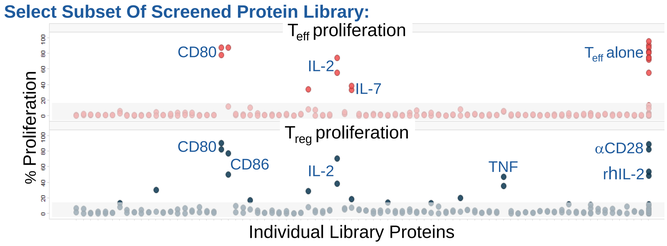
- Engineered K562 artificial antigen presenting cell:
  - Cell-surface anti-CD3 fragment to stimulate T cells
  - Non-signaling CD64 Fc receptor to capture library proteins
- Human pan T cells pre-activated, then rested
- Incubated with FivePrime library of extracellular domain (ECD) – Fc fusion proteins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



Regulatory T Cell Screen

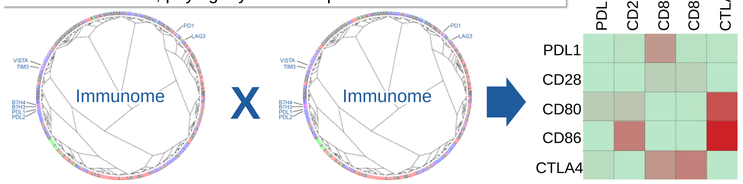


Select Subset Of Screened Protein Library:

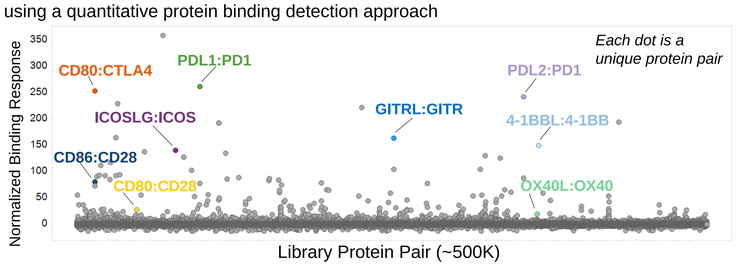


Combinatorial Receptor-Ligand Matching Screen

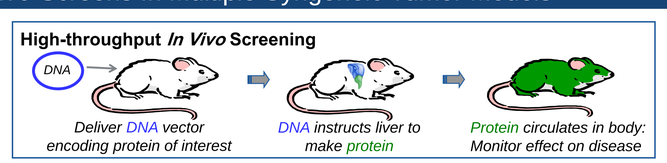
FivePrime 'Immunome': ~700 potential immune-regulatory proteins based on structure, phylogeny and/or expression



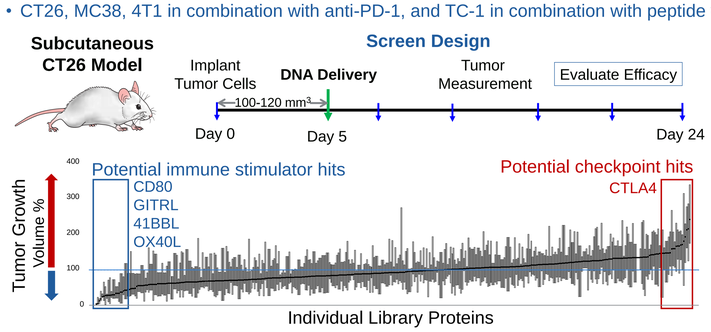
Screen goal: Identify novel protein-protein interactions within the FivePrime Immunome using a quantitative protein binding detection approach



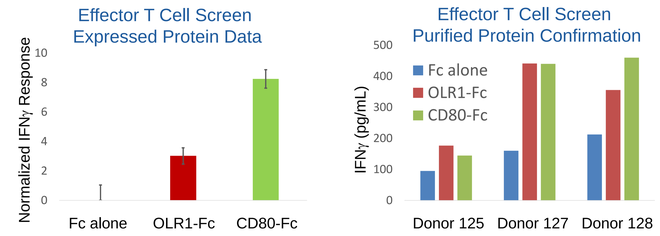
In Vivo Screens In Multiple Syngeneic Tumor Models



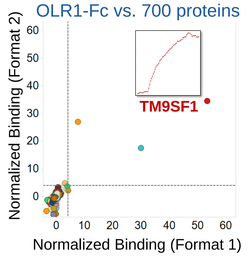
- Screened a library subset in 4 syngeneic tumor models with unique immune cell infiltrate profiles:
  - CT26, MC38, 4T1 in combination with anti-PD-1, and TC-1 in combination with peptide vaccine



Oxidized Low-Density Lipoprotein Receptor 1 (OLR1) Is A Novel T Cell Co-Stimulator For Which We Identified Novel Binding Partners

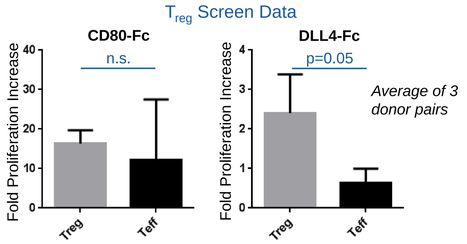


Immunome x Immunome data:
OLR1-Fc vs. 700 proteins



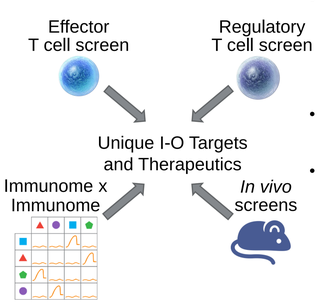
- OLR1 is known to be expressed on vascular endothelial cells and bind oxidized LDL, leading to pro-inflammatory responses
- We found that OLR1-Fc could co-stimulate effector T cells, a novel activity for this protein
- We also identified several novel interactions with OLR1 in the Immunome x Immunome (IxI) screen
  - One novel binding partner, TM9SF1, is expressed on T cells
- OLR1 – TM9SF1 may be a novel T cell co-stimulatory pathway

Soluble Delta-Like Protein 4 (DLL4) Specifically Stimulates Regulatory T Cells & Controls Anti-Tumor Immune Response *In Vivo*



- DLL4 is known to bind to Notch receptors and regulate angiogenesis
- Notch receptor signaling is important for differentiation of helper T cells and plays a critical role in controlling T_{reg} effector functions
- We found that human DLL4-Fc stimulated the proliferation of T_{regs}, but had a minimal effect on T_{effs}
- Additionally, mouse DLL4-Fc strongly reduced CT26 tumor growth in WT mice, but not in SCID mice
  - DLL4-Fc's anti-tumor activity is dependent on T (and/or B) cells

Summary And Next Steps



- We are taking multiple approaches to identifying novel immune modulatory pathways
  - *In vitro* screens of Effector T cells and T_{regs}
  - 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 T_{eff} and Immunome x Immunome screens
  - Unexpected mechanisms from known immune modulators
    - For example DLL4 from the T_{reg} and *in vivo* screens
- Combining data from multiple screening approaches we have prioritized a series of new targets for validation as potential novel immuno-oncology therapeutics