Identification of Novel Immune Regulators of Tumor Growth Using RIPPS^{SM} Screening *in vivo*

Tom Brennan, David Bellovin, Jacqueline De La Torre, Nebiyu Wondyfraw, Servando Palencia, Ernestine Lee, Kevin Hestir
Five Prime Therapeutics, Inc., South San Francisco, CA

Abstract

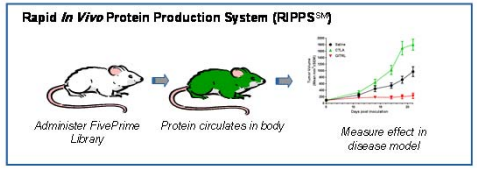
Identification of novel targets in cancer immunotherapy is needed to address the significant number of patients that either do not respond to current therapies or encounter unacceptable toxicities. The discovery of such targets, including novel checkpoint regulators and the counter-receptors for previously "orphan" checkpoints, has been limited by a lack of a comprehensive collection of proteins suitable for functional screening and methods for assessing their function in high-throughput.

We have generated a comprehensive library of substantially all human extracellular proteins, encompassing nearly every target for protein therapeutics. Our library contains more than 5700 proteins, including secreted protein ligands and the extracellular domains of membrane-bound receptors in soluble forms. The library proteins represent therapeutic targets and in some cases may act as therapeutics themselves. A portion of this library we call the immunome contains ~700 proteins with structural features characteristic of immune-activators and checkpoints that we selected.

RIPPS^{SM} technology is a robust method whereby FivePrime's library of soluble secreted proteins can be tested *in vivo* in virtually any mouse disease model. Each cDNA representing a unique protein is administered to a cohort of mice and results in high circulating levels of the encoded protein. RIPPS^{SM} also allows us to rapidly confirm activity identified by other *in vitro* screening approaches. Here, we have exploited RIPPS^{SM} technology to screen for new immuno-oncology therapeutics and targets for therapeutic development. As positive controls we performed RIPPS^{SM} on CT-26 tumor-bearing mice using known agonists and antagonists of the immune response, which resulted in decreased or increased tumor growth, respectively. Subsequently, we screened 350 immunome proteins by RIPPS^{SM} in the CT-26 tumor model and have identified proteins that enhance and inhibit tumor growth and display changes in TIL (tumor-infiltrating lymphocyte) profiles. These data demonstrate the power of our discovery platform to discover and validate novel therapeutic targets and protein therapeutics for immuno-oncology.

Background

The RIPPS^{SM} System



- Net result is same as high continuous infusion of purified recombinant protein
  - Achieves high blood levels for weeks after administration
- Compatible with any mouse model of disease
- Ideally suited for interrogating the function of secreted proteins and receptors

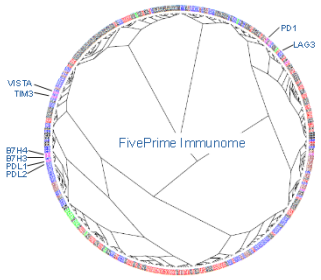
Results

FivePrime Prioritized the Immunome for RIPPS Screening to Identify Novel Immune Regulatory Molecules

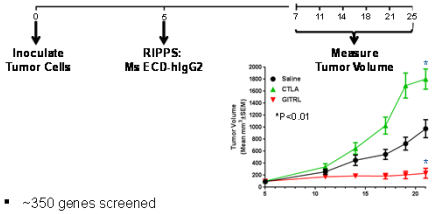
FivePrime Immunome

- Known immune checkpoints possess a combination of IgC, IgV, and ITIM/ITSM motifs.
- Our library was queried for any genes that may possess these motifs.
- Many of the genes in this "immunome" set cluster around known checkpoints.

Red = IgC+ITIM
Brown = IgV+ITIM
Blue=IgV
Green=ITIM only
Black=IgC
Magneta = Selected Genes

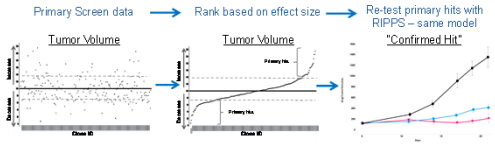


FivePrime Tested 350 Genes from the Immunome Collection in the CT-26 Tumor Model Using RIPPS Technology

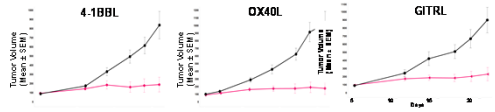


- ~350 genes screened
- Most genes were screened as Fc fusion constructs
- RIPPS administered when tumors reach ~100 mm^3
- CTLA4-IgG and GITRL-IgG served as controls for growth promoting and inhibiting properties, respectively
- Six genes have displayed novel tumor growth modulating activity

RIPPS Screen Workflow in the CT-26 Model



Several Genes with Known Anti-tumor Activity Were Randomly Placed in the Screen Queue and Show Activity

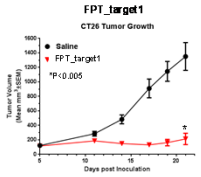


- CT26 tumor cells were inoculated subcutaneously onto the hindflank
- RIPPS was administered on day 5 after tumor inoculation
- 4-1BBL, OX40L, and GITRL were expressed as Fc-fusion proteins

Results

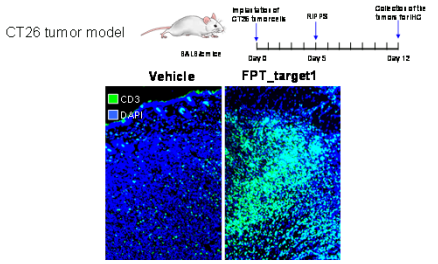
RIPPS Technology in the CT-26 Model has Revealed Multiple Gene Targets with Tumor-Modulating Activity

Name	Effect Size	P value
GITRL	Decreased 100%	P<0.01
FPT_target1	Decreased 80-100%	P<0.01
FPT_target2	Decreased 100%	P<0.01
FPT_target3	Decreased 80%	P<0.01
FPT_target4	Decreased 30%	P<0.05
FPT_target5	Increased 30%	P<0.05
FPT_target6	Increased 30%	P<0.05



- Multiple genes have produced anti-tumor responses
- Two genes have produced 100% effect sizes, similar to GITRL
- Two genes have resulted in accelerated tumor growth, similar to CTLA4-Fc

RIPPS of FPT_target1 Results in an Increase in CD3+ Tumor Cells by Day 7 After Treatment



- FPT_target1 results in dramatic increase in CD3+ cells into the tumor
- FPT_target1 displays synergistic activity with PD1 blockade (data not shown)
- FPT_target1 was not effective in SCID mice implicating an immune-mediated effect
- FPT_target1 is being evaluated for use as a therapeutic target for treatment of solid tumors

Conclusions

- RIPPS produces high circulating levels of protein from DNA encoding genes of interest
- RIPPS was used to screen 350 "immunome" genes in the CT-26 tumor model
- FivePrime identified several genes with novel tumor-inhibiting or tumor-promoting activities
- One of the RIPPS-expressed gene targets has been evaluated further and displays strong CD3 infiltrate activity into the tumor and also displays synergistic activity with PD1 blockade
- Additional studies are being conducted on each target in other tumor models and in combination with known immune-modulating drugs