FROM CODE TO CURE: Computational Identification, Functional Characterization and Antibody Blockade of a New Immune Checkpoint in the TIGIT Family of Interacting Molecules

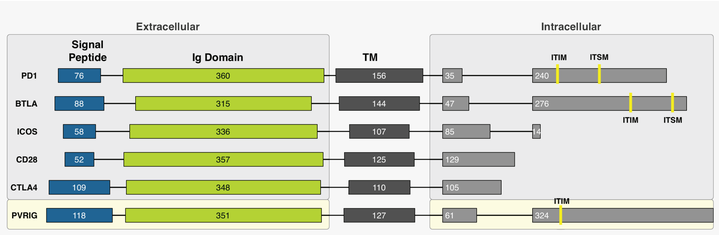
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Introduction

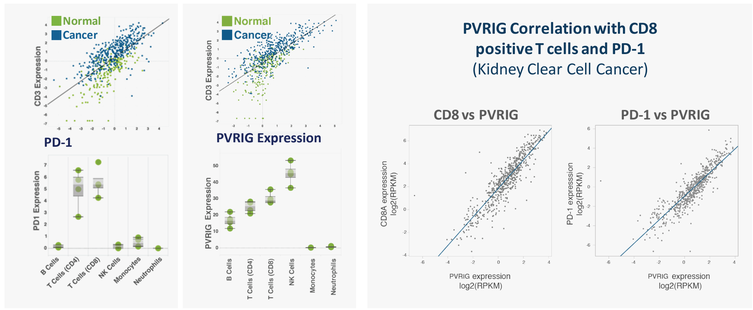
- **Background:** While antibody blockade of the CTLA4 and PD1 pathways has emerged as an effective treatment modality for cancer, the majority of patients do not derive long term benefit, suggesting a need for targeting of additional immune checkpoints. Employing our unique computational algorithms to define new members of the B7/CD28 family we identified PVRIG, which is expressed by multiple subsets of T and NK cells. We report here its expression pattern, functional characterization, and anti-tumor activity of blocking antibodies targeting this molecule.
- **Materials and Methods:** Utilizing Compugen's Predictive Discovery platform we identified PVRIG as a potential novel immune checkpoint, after which a retroviral cell screening library was used to identify its cognate binding counterpart. Target effects on T-cell modulation were assessed with primary and tumor-derived T-cell assays, taking advantage of target overexpression, knockdown, and antagonist antibody approaches. Antibodies against the human protein were screened for their ability to enhance T-cell activation in vitro, while antibodies targeting the mouse orthologue were assessed in vivo for effects on tumor growth inhibition in syngeneic models.

PVRIG FUNCTIONAL GENE STRUCTURE MATCHES KNOWN IMMUNE CHECKPOINT RECEPTORS



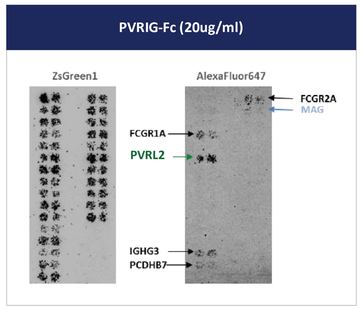
Use of 'Functional Homology' in absence of sequence similarity based on exon size, phase, and functional elements within exons

PVRIG EXHIBITS TUMOR EXPRESSION CHARACTERISTICS CONSISTENT WITH T-CELL RECEPTOR CHECKPOINTS

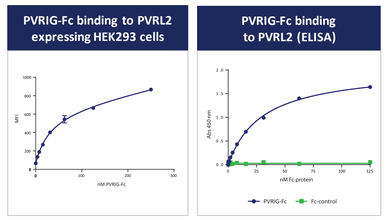


IDENTIFICATION OF PVRL2 AS THE LIGAND FOR PVRIG

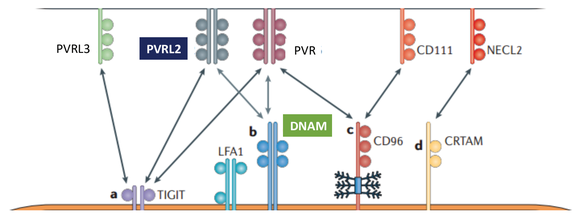
Retrogenix Cell Microarray



CONFIRMATION OF PVRIG BINDING TO PVRL2

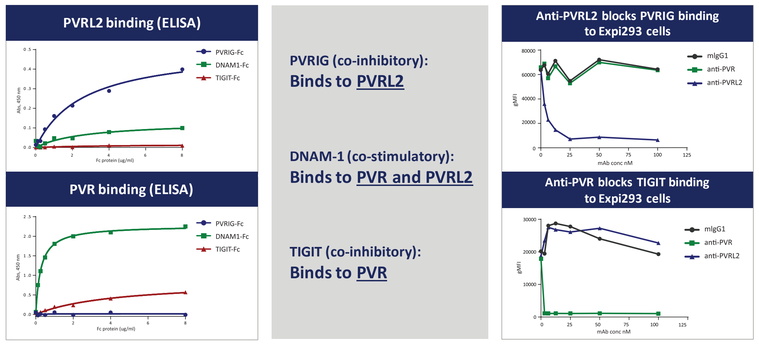


PVRL2 IS A LIGAND IN THE DNAM-1/TIGIT IMMUNE CHECKPOINT AXIS

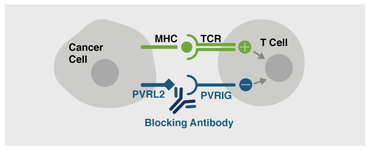


Martinet & Smyth, 2015 (modified)

BINDING INTERACTIONS IN THE PVRIG/TIGIT/DNAM AXIS



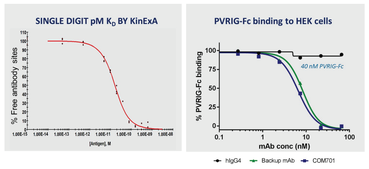
DEVELOPMENT OF COM701: A HIGH AFFINITY PVRIG ANTAGONIST



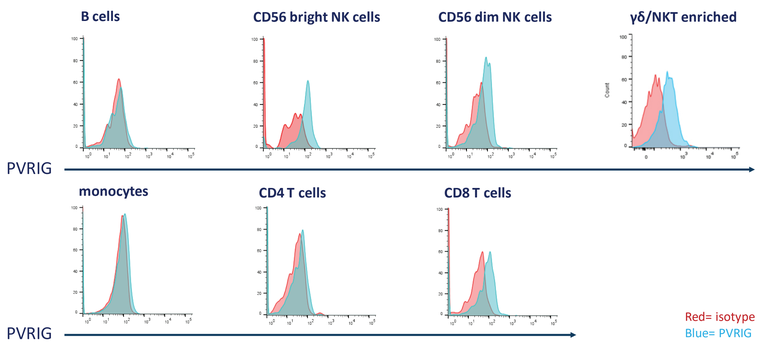
Human phage display and standard hybridoma

Antibodies screened for:
- High affinity (K_D < 1nM)
- Ability to block PVRIG/PVRL2 binding
- In vitro enhancement of T-cell activation

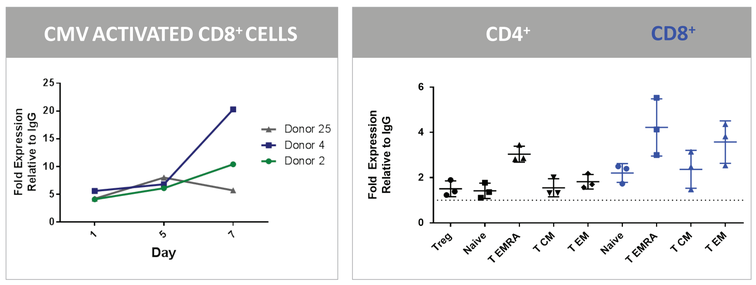
COM701 selected as therapeutic lead
IND anticipated in 2017



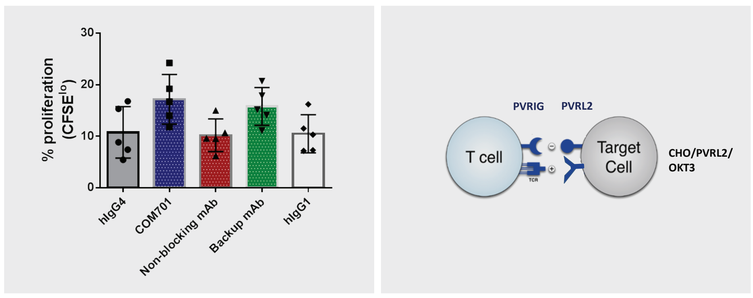
PVRIG EXPRESSION ON NAÏVE PBMC SUBSETS



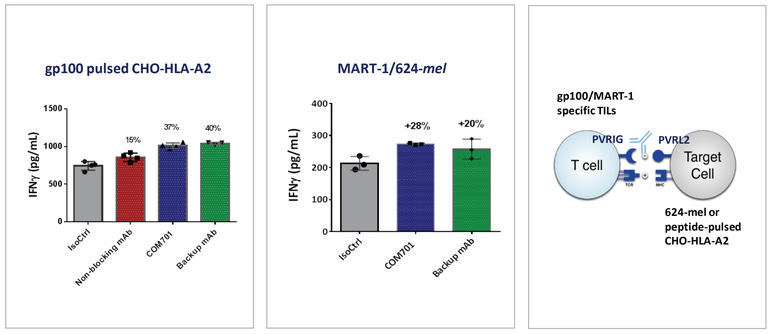
PVRIG EXPRESSION IS INDUCED FOLLOWING T-CELL ACTIVATION AND ELEVATED ON T_{EMRA} AND T_{EM} CELLS



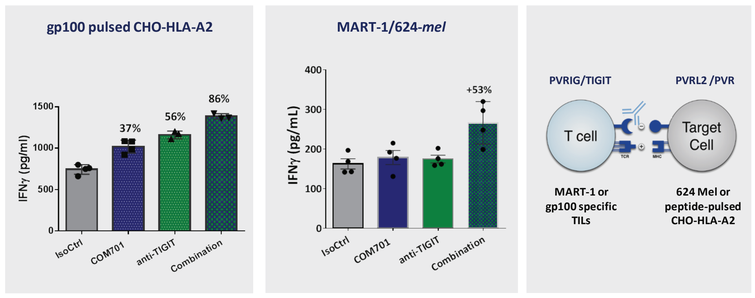
ANTAGONIST PVRIG ANTIBODIES INCREASE CD4+ T-CELL PROLIFERATION



ANTI-PVRIG BLOCKING ANTIBODIES ENHANCE TIL ACTIVATION

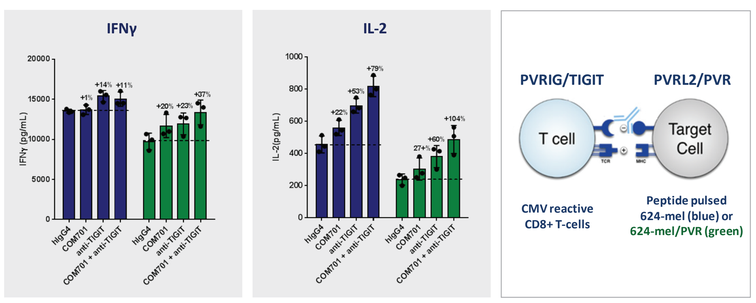


COMBINING PVRIG AND TIGIT BLOCKADE INCREASES TIL ACTIVATION

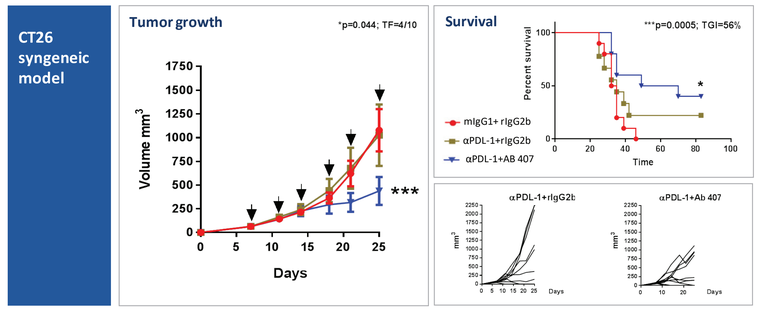


Compugen In house

COMBINING PVRIG AND TIGIT BLOCKADE RESULTS IN ENHANCED ACTIVATION OF CMV REACTIVE CD8+ CELLS



PVRIG BLOCKING ANTIBODIES REDUCE TUMOR GROWTH AND INCREASE SURVIVAL IN COMBINATION WITH PD1 PATHWAY BLOCKADE



SUMMARY

- **Results:** A PVRIG-Fc-fusion protein was found to bind PVRL2, with binding specificity confirmed both by ELISA and flow cytometry analysis. PVRIG demonstrated unique expression kinetics upon T-cell activation, with detection of the target on memory T-cells, as well as on NK cells and γδ T-cells. A panel of high affinity human antibodies with the ability to block interaction of PVRIG with PVRL2 were generated, which when tested in vitro were shown to enhance activation of both primary CD4+ and tumor-derived CD8+ T-cells through a PVRL2-dependent mechanism. The lead antibody, COM-701, is currently in preclinical development.
- Since COM-701 is not mouse cross-reactive, in vivo studies were conducted with a surrogate blocking anti-mouse PVRIG antibody. When combined with anti-PDL1 blockade, anti-mouse PVRIG inhibits growth of established tumors in both the CT26 and MC38 colorectal cancer models. Combination testing with additional immune checkpoint inhibitors, as well as in PVRIG knockout mice, is ongoing.
- **Conclusion:** We describe the identification of PVRIG as a novel immune checkpoint on T cells, as well the development of a high affinity antagonistic antibody, COM-701, that is currently in preclinical development. COM-701 is able to enhance human T-cell activation, and a surrogate antibody with similar characteristics shows synergy with PD-L1 in vivo in multiple syngeneic models. Overall, our data demonstrates the utility of targeting PVRIG in addition to other B7 family checkpoints for the treatment of cancer.