Protein Engineering by Directed Evolution to Derive ALPN-101, a Dual ICOS/CD28 Antagonist ICOSL Variant Ig Domain (vIgD)-Fc Fusion Protein for the Treatment of Inflammatory Diseases

Mark Rixon, Ph.D.
Sr. Director, Protein Therapeutics

2019 Protein Engineering Summit

Forward Looking Statements

This release contains forward-looking statements within the meaning of Section 27A of the Securities Act of 1933, Section 21E of the Securities Exchange Act of 1934 and the Private Securities Litigation Reform Act of 1995. These forward-looking statements are not based on historical fact and include statements regarding our platform technology and potential therapies, the timing of and results from clinical trials and pre-clinical development activities, the potential efficacy, safety profile, future development plans, addressable market, regulatory success and commercial potential of our product candidates, the efficacy of our clinical trial designs and our ability to successfully develop and achieve milestones in our development programs. Forward-looking statements generally include statements that are predictive in nature and depend upon or refer to future events or conditions, and include words such as “may,” “will,” “should,” “would,” “expect,” “plan,” “intend,” and other similar expressions among others. These forward-looking statements are based on current assumptions that involve risks, uncertainties and other factors that may cause actual results, events or developments to be materially different from those expressed or implied by such forward-looking statements. These risks and uncertainties, many of which are beyond our control, include, but are not limited to: clinical trials may not demonstrate safety and efficacy of any of our product candidates; our ongoing discovery and pre-clinical efforts may not yield additional product candidates; our discovery-stage and pre-clinical programs may not advance into the clinic or result in approved products; any of our product candidates may fail in development, may not receive required regulatory approvals, or may be delayed to a point where they are not commercially viable; we may not achieve additional milestones in our proprietary or partnered programs; the impact of competition; adverse conditions in the general domestic and global economic markets; as well as the other risks identified in our filings with the Securities and Exchange Commission. These forward-looking statements speak only as of the date hereof and we undertake no obligation to update forward-looking statements, and readers are cautioned not to place undue reliance on such forward-looking statements.

Alpine Immune Sciences, Inc.

- Founded 2015 in Seattle , WA
- Core Technology: Variant Immunoglobulin Domain (vIgD™) platform
- Clinical stage company including:
  - Discovery and Translational Sciences
  - Protein Engineering and Therapeutics
  - CMC
  - Clinical
- Developing novel therapies for cancer and autoimmune/inflammatory diseases

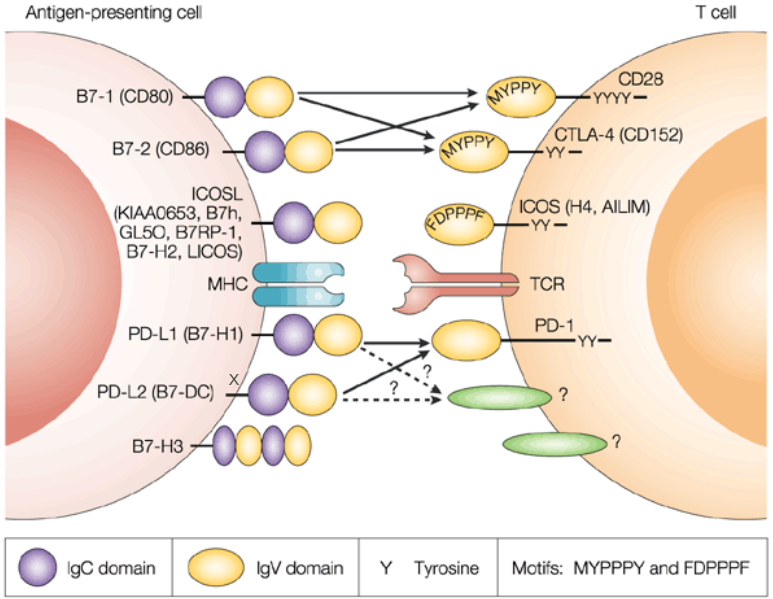
Immunoglobulin Superfamily (IgSF) Proteins Present Attractive Targets for the Generation of Novel Therapeutics

- IgSF is the largest human protein superfamily – 765 members identified[1]
- Named for the Ig domain (70-110 amino acids) also found in antibodies
- Includes many targets which have evolved as critical regulators of immunity, e.g.:

Group	Examples
Checkpoint	PD-1, PD-L1, CTLA-4, TIGIT, Lag-3, VISTA, CD47
Costimulatory	CD28, ICOS, CD80, CD86, CD2
Antigen Receptor-Related	CD3, TCR, BCR, MHC, CD19, CD4, CD8
Cytokine Receptors	IL-1R, IL-6R, CSF1R

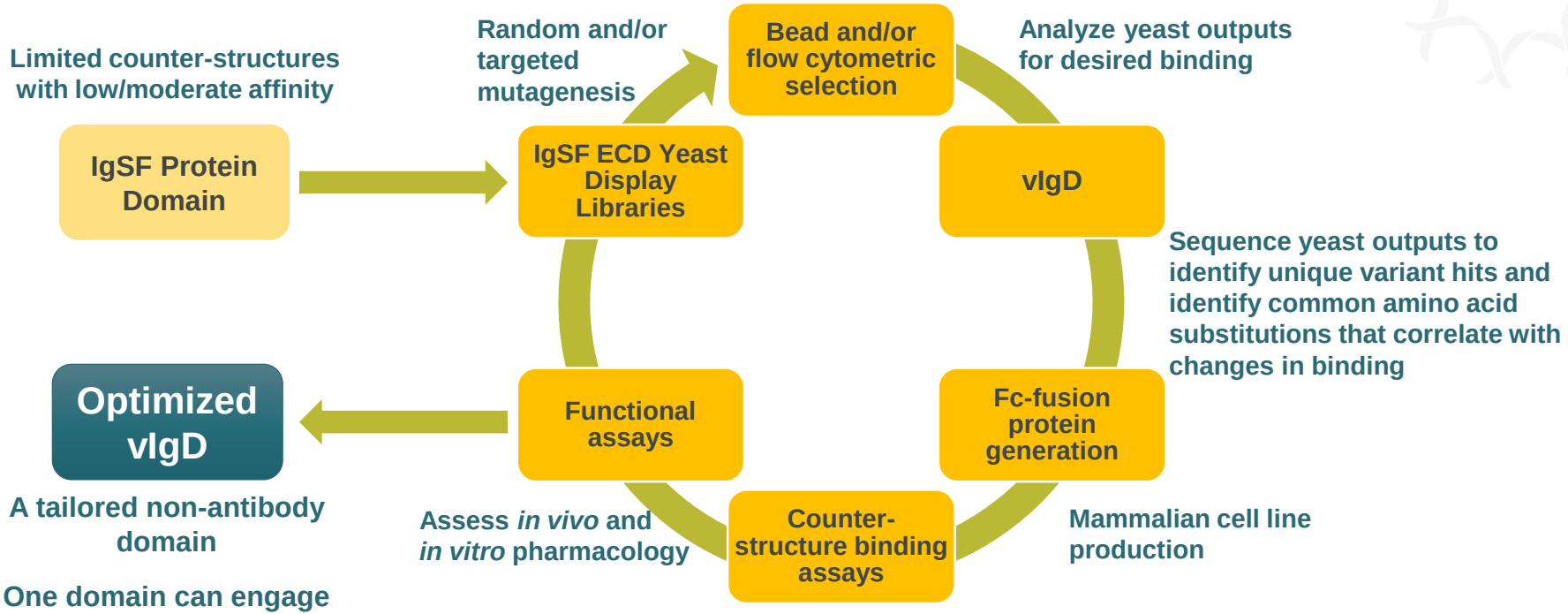
[1]Nature 409 :860 (2001)

The CD28 Protein Family
A Critical IgSF Subfamily



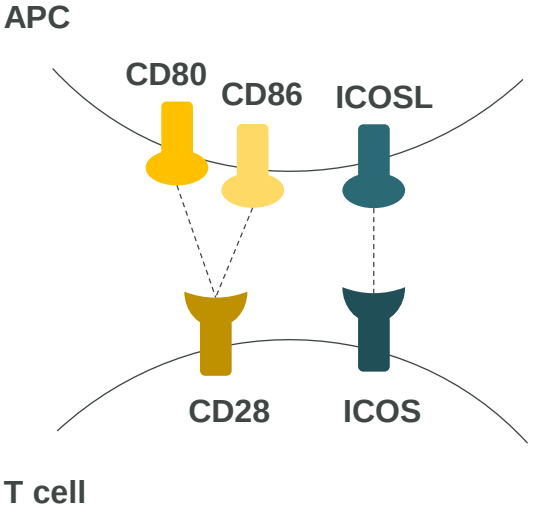
Nat Rev Immunol 2:116 (2002)

Variant Ig Domains (vIgDs) are IgSFs Engineered via Directed Evolution to Acquire Target Specificity and Optimize Immune Modulation



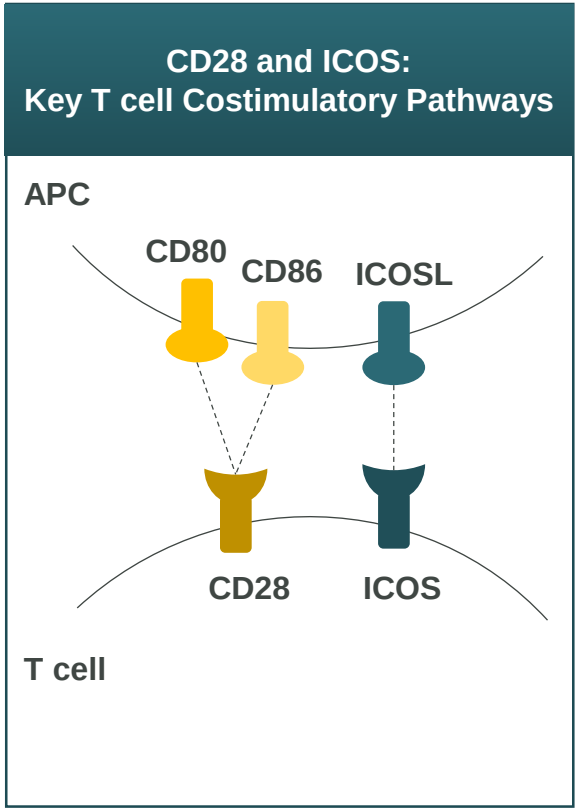
Application of vIgD Platform to the Discovery and Engineering of a Therapeutic Candidate

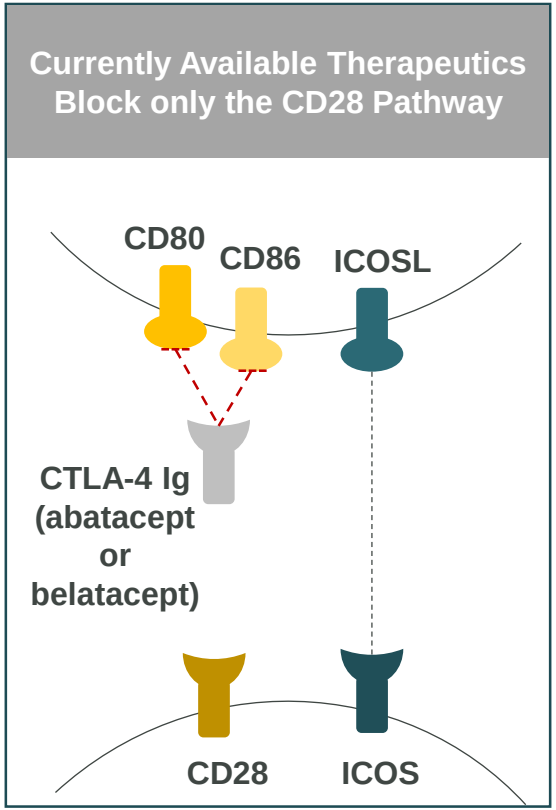
CD28 and ICOS:
Key T cell Costimulatory Pathways



- CD28 and ICOS pathways mediate separate T cell costimulatory pathways
- CD28 and ICOS bind CD80/CD86 and ICOS ligand (ICOSL), respectively and play critical roles in T cell activation and adaptive immunity
- In preclinical inflammatory conditions neutralizing only one of these pathways is not sufficient for effective disease suppression

CTLA-4 Ig Only Blocks the CD28 Costimulatory Pathway

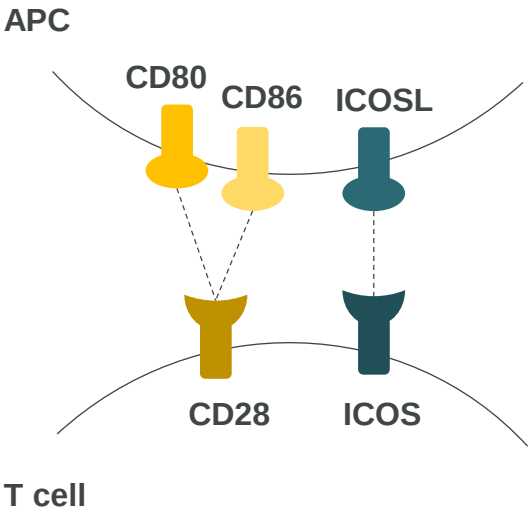




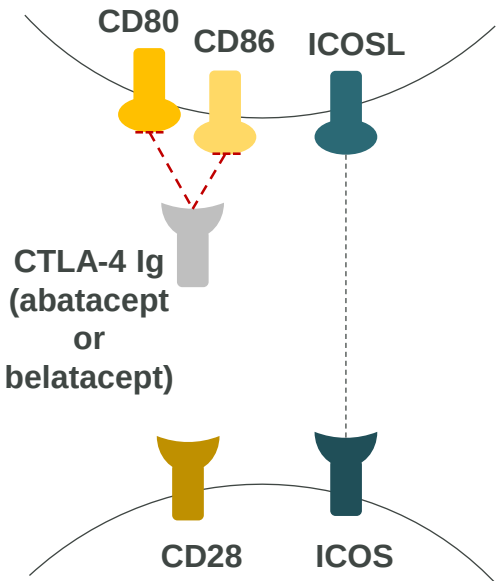
- Following antigen encounter, most activated T cells become CD28+ ICOS+, but a subset downregulate CD28
- CD28- ICOS+ cells are resistant to CTLA-4-Fc therapy and appear to play important roles in inflammatory and autoimmune diseases
- Blocking only one pathway is likely insufficient to effectively inhibit T cell activation

A Dual CD28/ICOS Antagonist Will Block both CD28 and ICOS Costimulatory Pathways

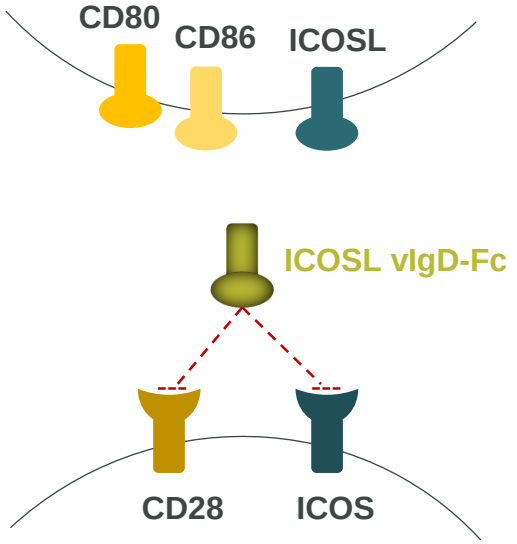
CD28 and ICOS: Key T cell Costimulatory Pathways



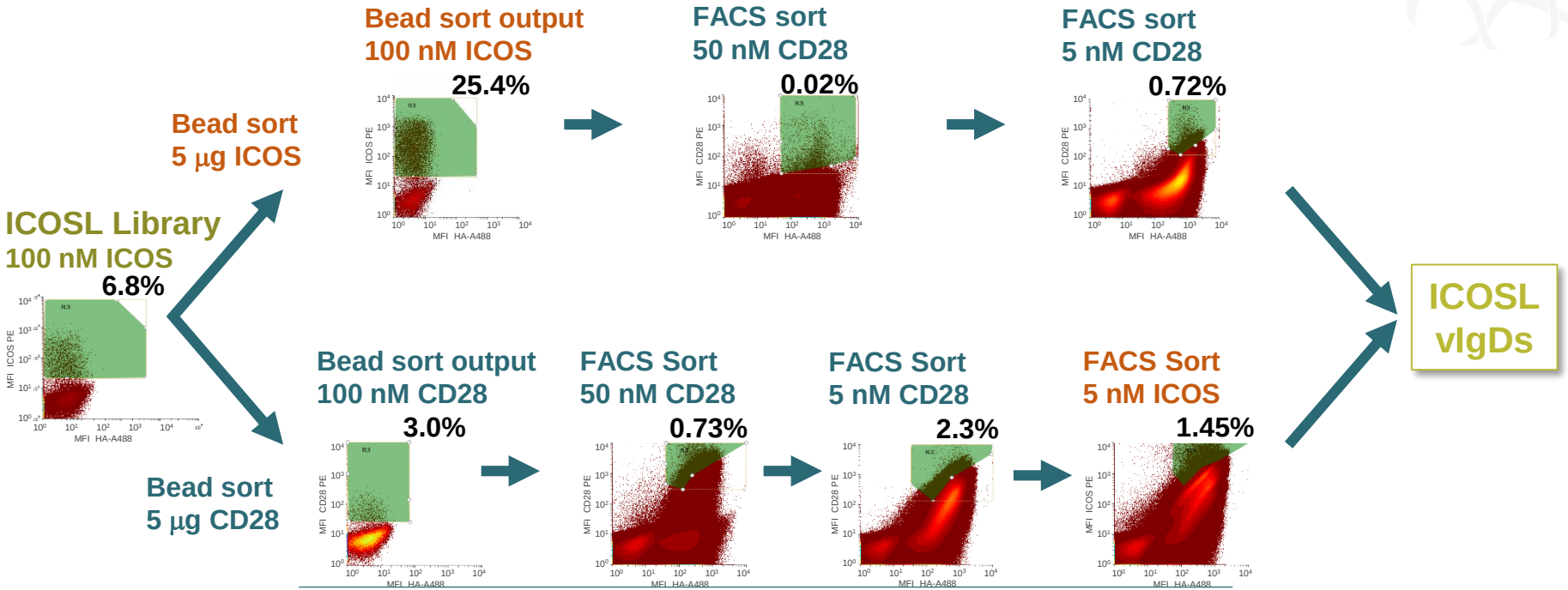
Currently Available Therapeutics Block only the CD28 Pathway



ICOSL vIgD-Fc Blocks both the CD28 and ICOS Pathways

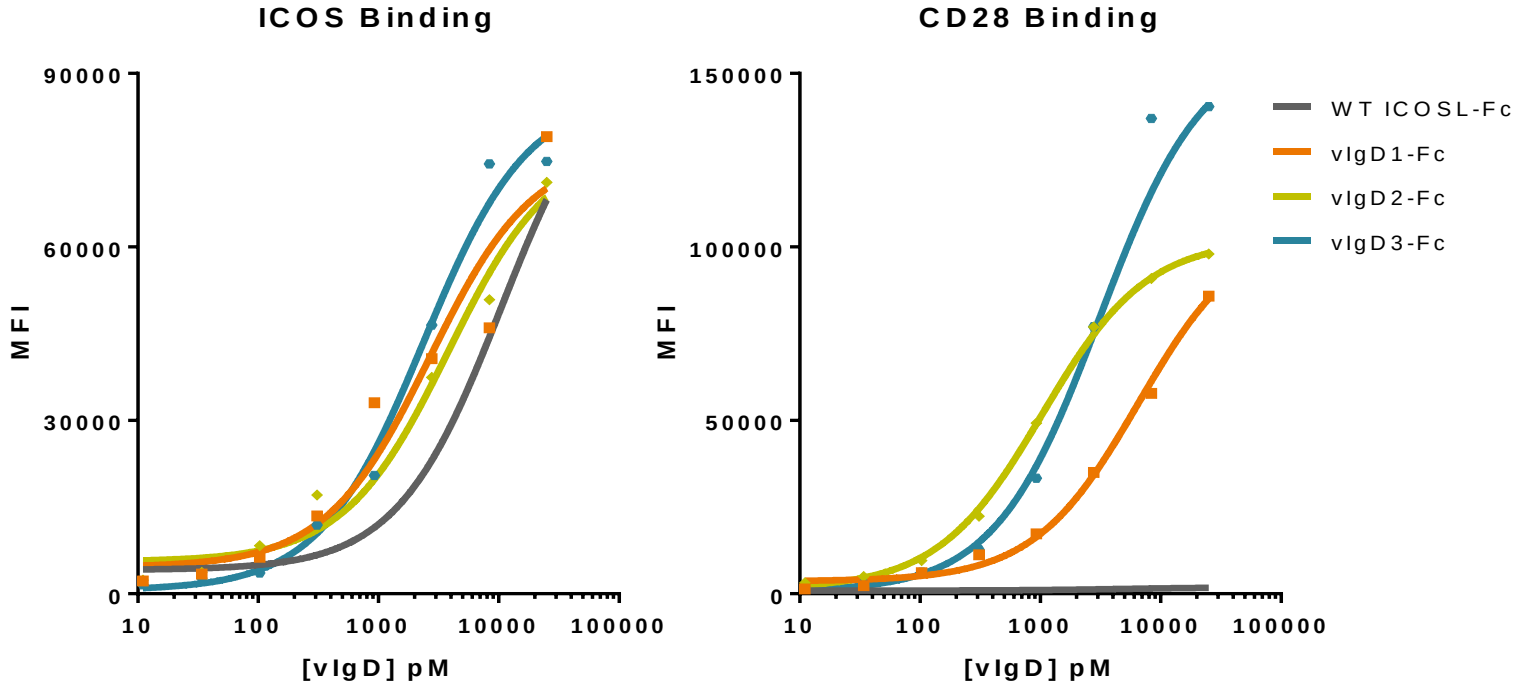


Selection of Mutated ICOSL Yeast Display Library Against Decreasing Concentrations of ICOS and CD28 Yields Panel of High-Affinity, Dual-Specific ICOSL vIgDs



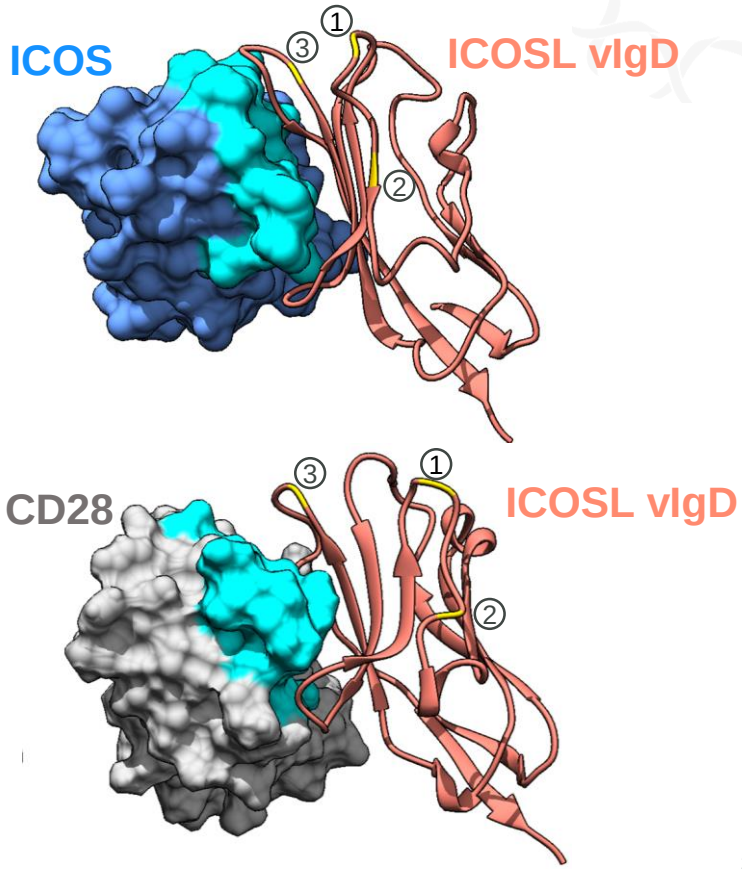
|<----- Flow Cytometry Binding ------>|<---------- Flow Cytometry Sorting ---------->|

Conversion of ICOSL vIgDs into Fc Fusion Proteins Maintains Enhanced Dual Binding Activity to Counter-Structures

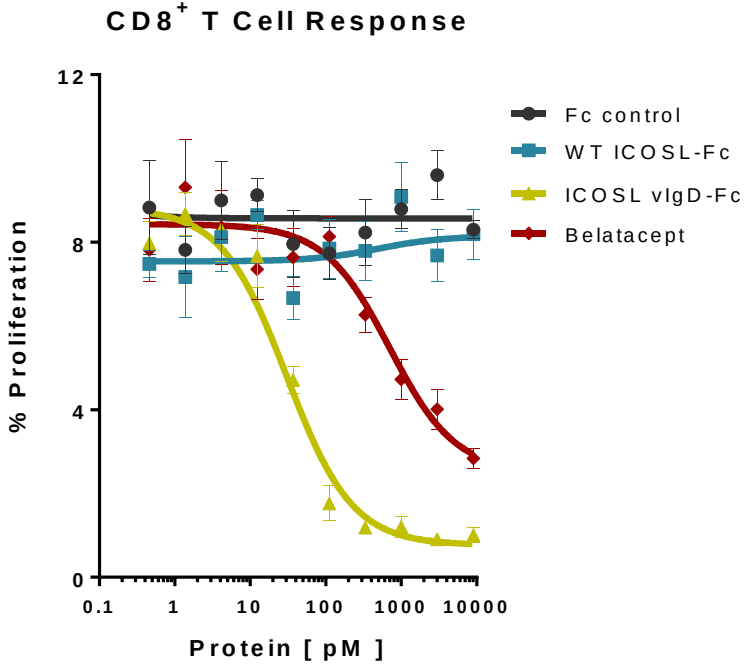


Structural Modeling of Binding Interfaces of ICOSL vIgD and the Convergence of Directed Evolution in Three Key Positions

- Low number of amino acid substitutions required for binding affinity improvements
- The set of highest affinity ICOSL vIgDs contained amino acid substitutions at similar residue positions
- Amino acid substitution positions located at, and distant from, modeled binding interfaces
- Substitutions converged on similar amino acid classes at each position
  - ① and ③ favored positively charged residues
  - ② favored non-polar aromatic residues



ICOS/CD28 Dual Antagonist ICOSL vIgD-Fc Demonstrates Potent T Cell Inhibition in Human Mixed Lymphocyte Reaction

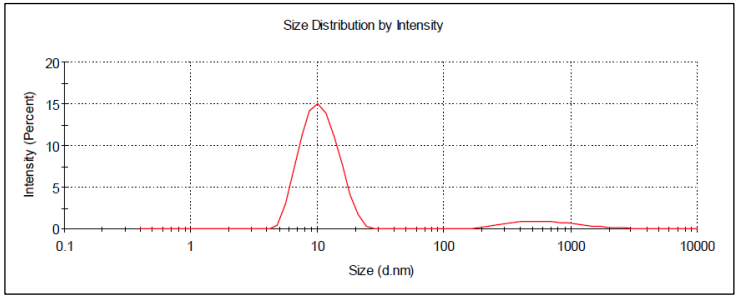


T cell Subset	Proliferation IC_{50} (pM)		
	WT ICOSL-Fc	ICOSL vIgD-Fc	Belatacept
$CD8^+$	-	30.5	704.6

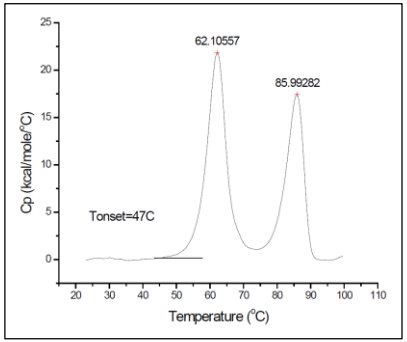
- ICOSL vIgD-Fc demonstrates improved immunomodulatory activity relative to CD28 inhibition alone

ICOSL vIgD-Fc Final Lead Candidate Possesses Biophysical Properties Amenable for Large Scale Manufacture

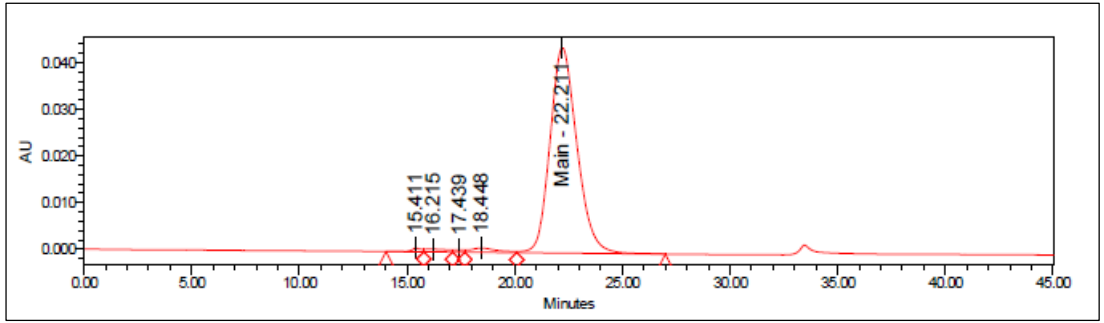
Dynamic Light Scattering - monodisperse



Differential Scanning Calorimetry
T_m profile similar to an IgG



Analytical Size Exclusion Chromatography-HPLC - homogeneous



ICOSL vIgD-Fc Manufacturability

- High titer from stable CHO clones
- High protein homogeneity
- Good stability profile
- Lot-to-lot consistency

Optimization of Lead ICOSL vIgD-Fc Fusion Proteins and Selection of Clinical Candidate ALPN-101

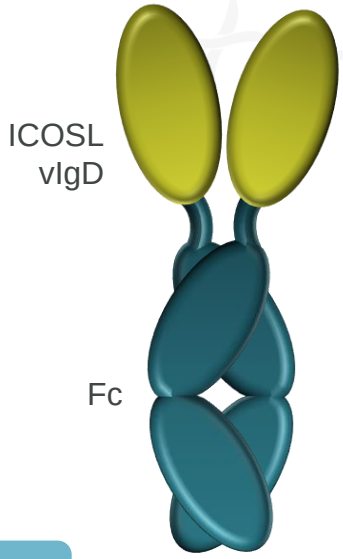
vIgD candidates selected for desired counter-structure binding
- Maintain high affinity to ICOS
- Introduced high affinity to CD28

vIgD-Fc fusion proteins selected based on transient expression productivity and protein quality

Fc selected with reduced/ablated effector function activity

Linker length and ICOSL fusion point selected based on functional bioactivity

Manufacturability assessed by expression productivity and protein quality from stable CHO cells

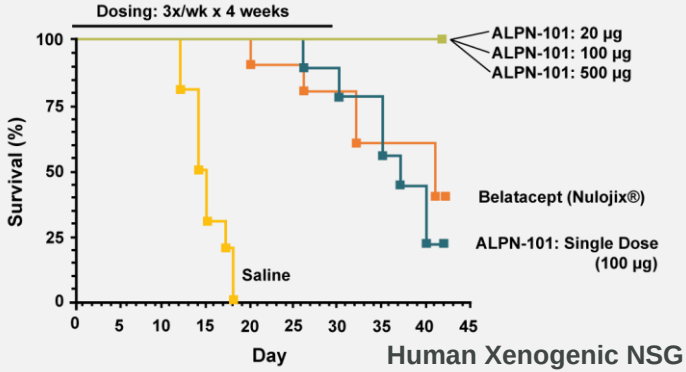


ALPN-101

ALPN-101: Broad Potential in Multiple Therapeutic Areas

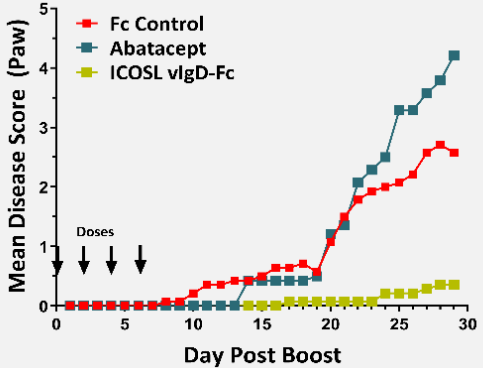
Superior Efficacy in Diverse Preclinical Models vs. Active Comparators

Heme/Onc: Acute Graft-Versus-Host Disease



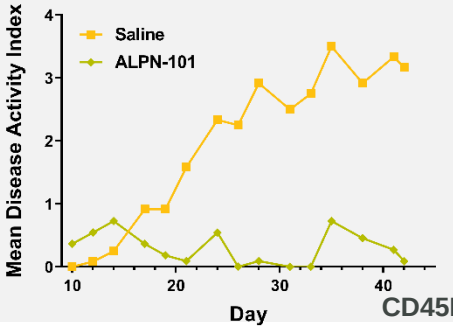
Human Xenogenic NSG

Rheumatology: Rheumatoid, Psoriatic



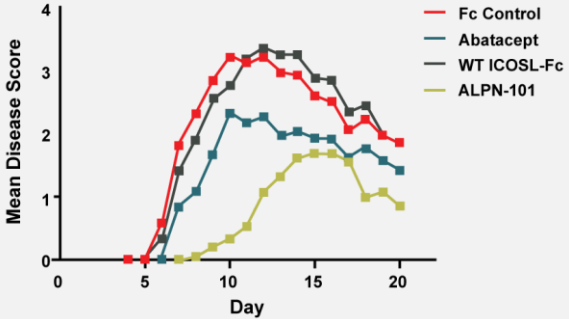
Collagen-Induced Arthritis

GI: Inflammatory Bowel Disease



CD45RBhi Colitis

Neurology: Multiple Sclerosis



MOG Adoptive Transfer Experimental Autoimmune Encephalomyelitis

Summary

- Alpine Immune Sciences' vIgD platform engineers IgSF domains via directed evolution to increase target affinity, acquire new target specificity and optimize immune modulation
- The vIgD platform was utilized to generate dual specific molecules with enhanced binding to ICOS and novel binding to CD28
- ALPN-101 is a novel immuno-modulatory ICOSL Fc-fusion protein therapeutic candidate with optimized affinity to ICOS and CD28;
  - Demonstrates potent functional activity *in vitro* and potent efficacy *in vivo*
  - Possesses bioanalytical characteristics consistent with scalable manufacture
  - Has completed manufacturing campaigns to support early clinical trials
  - Has shown efficacy in pre-clinical development for a variety of autoimmune and inflammatory disease and has entered clinical development

The Alpine Immune Sciences' vIgD platform is able to generate multispecific IgSF-based therapeutic candidates not only applicable in inflammation, but potentially applicable in cancer, infectious disease, and endocrinological and neurological disorders.

Acknowledgements

Protein Engineering/Therapeutics

- Janhavi Bhandari
- Irene Capuano
- Dan DeMonte
- Logan Garrett
- Luanne Hebb
- Michael Kornacker
- Joe Hoover
- Sean MacNeil
- Andrea Peng
- Martin Wolfson

Translational Sciences

- Stacey Dillon
- Katherine Lewis
- Paramita Mookherjee
- Sherri Mudri
- Kayla Susmilch
- Fariha Ahmed-Qadri

Bioanalytical Sciences

- Russell Sanderson
- Michelle Seaberg
- Kathy Henderson

Immunology

- Susan Bort
- Kelli Burley
- Lawrence Evans
- Chelsea Gudgeon
- Steve Levin
- Mark Maurer
- Chris Navas
- Erika Rickel
- Ryan Swanson

Alpine R&D Executives

- Kristine Swiderek
- Stanford Peng

ALPINEImmuneSciences

Thank You