Poster P343

Novel Immuno-Oncology Biologics Derived via Directed Evolution of IgSF Domains

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Abstract

Background: Our variant Ig domain (vIgD)™ platform creates novel, therapeutically-applicable protein domains with tailored specificity and affinity. These vIgDs are created through directed evolution of immunoglobulin superfamily (IgSF) proteins and have unique biochemical properties including small size, single domain structure, and the capacity to interact with multiple counter-structures. Because many IgSF family members and their counter-structures are widely expressed on immune cells and tumors, the vIgD platform is well positioned for the development of immuno-oncology biologics with potential first-in-class mechanisms of action. Here, multiple therapeutic formats for vIgDs were developed and characterized.

Methods: Novel vIgDs were created with tailored affinities and modulatory activities against PD-1, TIGIT, PD-L1, CTLA-4, CD28, and/or ICOS. These domains were successfully developed into multiple therapeutic formats, including single and multiple domain Fc fusion proteins and vIgD-monoclonal antibody (V-mAb) fusion proteins. In addition to ligand binding and specificity assays, *in vitro* functional activity was characterized in several T cell-based assays including cell-based reporter systems for pathway agonism or antagonism, primary human mixed lymphocyte reactions (MLRs), and costimulation assays utilizing artificial APCs (assessed by proliferation and IFNg production).

Results: Several functionally active therapeutic vIgD-based molecules were created successfully. (1) Single-domain vIgD-Fc fusion proteins with tailored binding to CD28, CTLA-4, and PD-L1 demonstrated differential activity in T cell activation assays and, depending on their ligand binding profile, resulted in greater or reduced IFNγ production and T cell proliferation in human T cell activation assays. (2) Multi-domain vIgD-Fc fusion proteins demonstrated promising targeting of immunomodulatory pathways in cell-based reporter assays and MLRs as assessed by IL-2 signaling and IFNγ production. Efficacy was comparable to monoclonal antibodies against the individual vIgD targets. (3) V-mAbs demonstrated target-specific T cell proliferation and IFNγ production in vitro, using both recombinant target proteins or target-specific cell lines.

Conclusions: The vIgD platform has successfully generated multiple immuno-oncology therapeutic candidates, in various formats including single- and multiple-domain Fc fusion proteins as well as V-mAbs. These varied formats confer, from a single molecule, multiple advantages including the multi-target modulation capability of evolved IgSFs, and, where applicable, tumor localizing capability of partner molecules or domains. This platform may contribute to the next generation of immunotherapeutic proteins in an oncology setting and efforts are ongoing to develop these candidates for human therapeutic use.

The Variant Immunoglobulin Domain (vIgD) Platform

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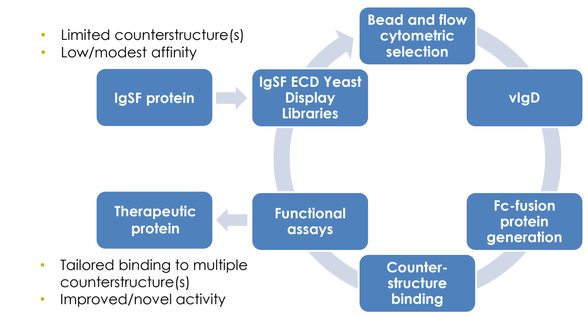
- Directed evolution: yeast maturation of individual IgSF domains
- Selection performed by flow cytometry
- Screening of mammalian cell-expressed domains via binding and functional assays
- Iterative process yields unique vIgDs evolved to modulate multiple counter-receptors

The immunoglobulin superfamily (IgSF):

- Each IgSF member consists of one or more 70-110 aa Ig domain
- Two types of Ig domains: Variable (IgV), constant (IgC1, IgC2)
- Key protein in the immune synapse e.g. PD-1/L1, CTLA4, TIGIT, CD28



The vIgD Platform: Multiple Therapeutic Formats

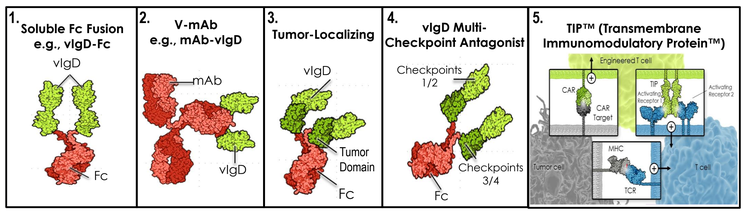


Fig. 1. vIgDs can be used in Multiple Therapeutic Formats.
- Single domains tailored for unique bi- or tri-specific binding and activity profiles (1)
- vIgDs can be fused to antibodies or other domains to provide site-directed T cell agonism (2,3)
- Fused vIgDs can bind two or more distinct targets i.e., bi- or tri-specific checkpoint inhibition (4)
- Cell-displayed for enhancement of adoptive therapies (5)

B7-CD28 Superfamily Tri-Specific vIgD Can Target Tumors with Multiple MOA

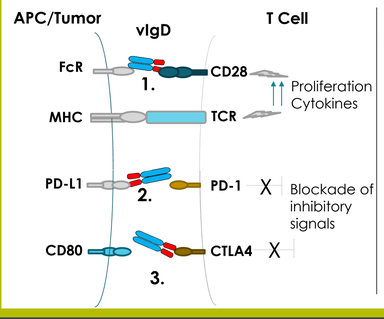


Fig. 2. Tri-Specific vIgD: Multiple Potential MOA:

1. Agonism of CD28 with FcR cross-linking
2. Blockade of PD-1/PD-L1 interaction
3. Blockade of CD80/CTLA4 interaction
4. Depletion of PD-L1-hi tumors
5. Depletion of CTLA4-hi Treg

B7-CD28 Superfamily Tri-Specific vIgD with Unique Binding & Activity Profiles

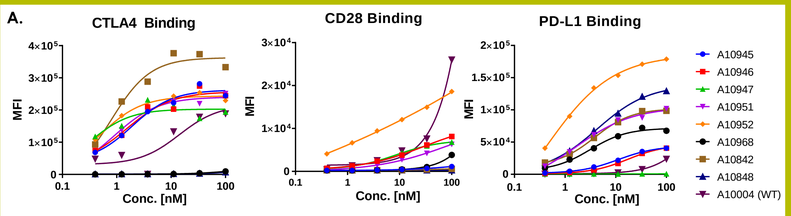
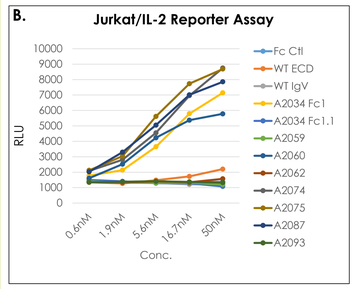


Fig. 3A. Tailored Binding to Multiple Counter-structures. vIgDs bind cell-surface CTLA4, CD28, or PD-L1 with unique profiles as measured by flow cytometry.



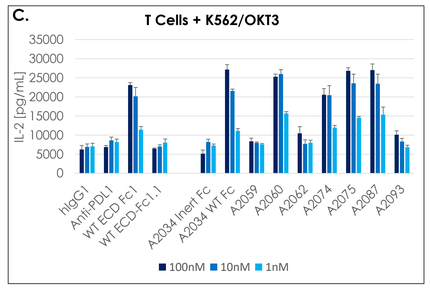


Fig. 3B. Tri-Specific vIgDs Can Induce CD28 Costimulation via FcR Cross-Linking. Jurkat/IL-2 luciferase reporter cells are mixed with CHO/OKT3 artificial APCs. Tri-specific vIgD-Fc proteins are then able to induce CD28 costimulation via Fc/FcR cross-linking on the APC. vIgDs with higher affinity for CD28 tend to produce a stronger CD28 costimulatory signal.

Fig. 3C. T Cell Stimulation with K562/OKT3 Artificial APCs. Primary human T cells are co-cultured with K562/OKT3 cells. The addition of tri-specific vIgDs with effector-function positive Fc provides CD28-mediated costimulation resulting in elevated IL-2 production. vIgDs with an inert Fc do not provide costimulation (A2034 Inert Fc).

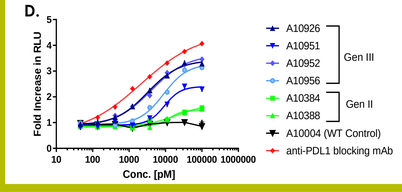
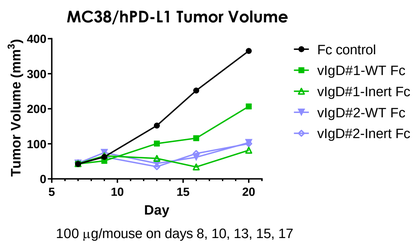


Fig. 3D. Tri-Specific vIgDs can Block PD-1/PD-L1 Interaction. Jurkat/IL2 reporter cells expressing PD-1 were incubated with CHO/OKT3/PD-L1 artificial APCs. Addition of tri-specific vIgDs blocked PD-1-mediated suppression of the TCR activation resulting in increased luminescence. Screening for increased PD-L1 binding affinity yielded better blockers in Generation III (Gen III) vIgDs compared to Generation 2 (Gen II).

B7-CD28 Superfamily Tri-Specific vIgDs Display Anti-Tumor Activity *In Vivo*

Fig. 4A. Tri-Specific vIgDs are Functionally Active *In Vivo*. Mouse MC38 tumor cells were stably transfected with human PD-L1 and implanted into C57BL/6 mice. Human tri-specific vIgDs were injected i.p. on days 8, 10, 13, 15 and 17 and tumor volume tracked over time. Suppression of tumor growth was observed in all vIgD treatment groups.



100 μg/mouse on days 8, 10, 13, 15, 17

V-mAbs → Adding T Cell Costimulation to Tumor-Specific mAbs

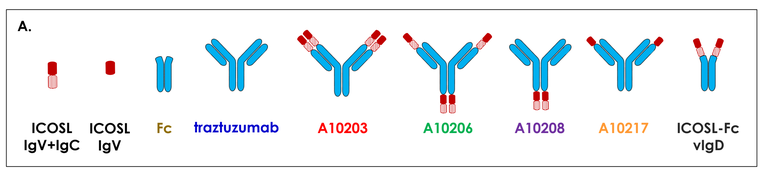


Fig. 5A. V-mAb Constructs. Due to their small size and modular nature, vIgDs can be fused to antibodies at several locations. Each of the V-mAbs depicted schematically were produced and tested for binding and function.

Fig. 5B. V-mAb Binding. V-mAb binding affinity to HER2 is slightly lower than parental trastuzumab. Affinity of ICOSL vIgD to CD28 or ICOS varies depending on site of antibody fusion.

Fig. 5C. Plate-Bound Costimulation. CD28-costimulation was measured when the V-mAb was plate-bound with anti-CD3. Level of V-mAb activity appears to be driven by avidity and spatial arrangement of the vIgD on the mAb.

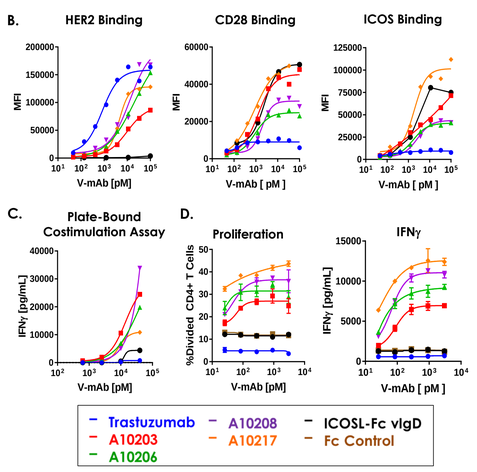
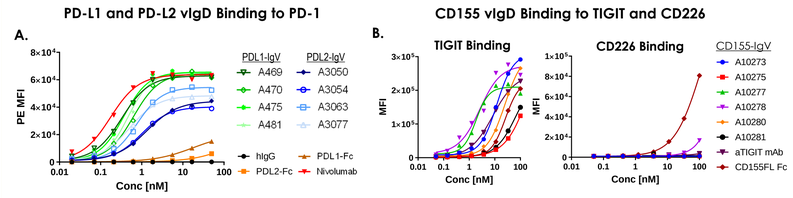
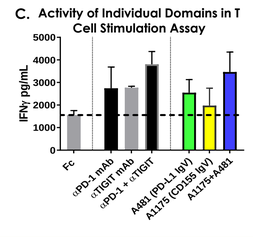


Fig. 5D. NCI-N87 Tumor-Mediated T-Cell Proliferation. NCI-N87 (HER2+) human gastric carcinoma cells were transduced with anti-CD3 single chain Fv (OKT3). Human pan T-cells were plated with tumor cells and V-mAb or control proteins. No effect above OKT3 stimulation observed with ICOSL-Fc as it is not co-localized with the TCR signal. Increased proliferation and cytokine production was observed with V-mAbs.

Characterization of Single-Domain Checkpoint-Inhibitory vIgDs

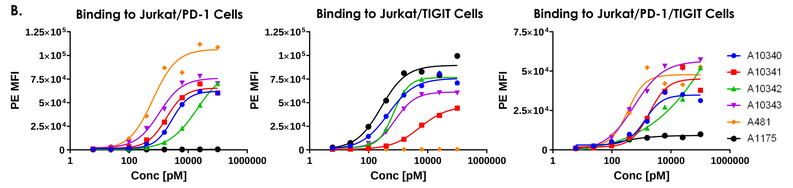


Fix. 6A. Identification of High Affinity PD-L1 and PD-L2 anti-PD-1 Domains. Individual PD-L1 IgV-Fc and PD-L2 IgV-Fc proteins were tested for binding to PD-1 transfectants by flow cytometry.
Fig. 6B. Identification of TIGIT-Specific CD155 Domains. CD155 IgV-Fc proteins were selected for high affinity for TIGIT and low affinity for CD226. Binding to CD96 and CD112R was also measured (not shown).
Fig. 6C. Functional Screening of Individual Domains. Selected domains were tested for capacity to block PD-1 and TIGIT mediated inhibitory signal in a T cell activation assay with CHO/OKT3/PD-L1/CD155 artificial APCs. Potent domains were selected for combination into fused vIgD Fc molecules.



Fused Multi-Domain vIgDs Bind Multiple Counter-Structures

Fig. 7A. Individual vIgDs were Combined into Fused-Domain Molecules. PD-L1-CD155 and PD-L1-CD155-CD112 molecules were generated, characterized for binding and ability to block PD-1 and TIGIT.



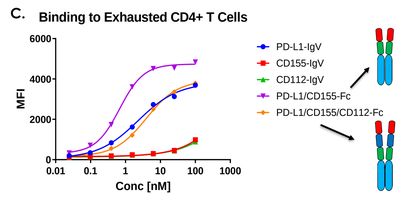


Fig. 7B. Fused vIgD Fc Binding to Jurkat Transfectants. PD-L1-CD155-Fc proteins were tested for binding to cells expressing either PD-1, TIGIT, or both. A successful multi-domain vIgD bound both PD-1 & TIGIT with high affinity.
Fig. 7C. Fused vIgD Binding to 'Exhausted' T Cells. Exhausted T cells were generated by three rounds of stimulation and expansion with K562/OKT3 + IL-2. PD-L1-CD155 and PD-L1-CD155-CD112 vIgD Fc were then tested for binding. PD-L1-CD155 vIgD Fc demonstrated superior binding to exhausted T cells than either domain alone.

Multi-Checkpoint vIgD Fusions are Functionally Active

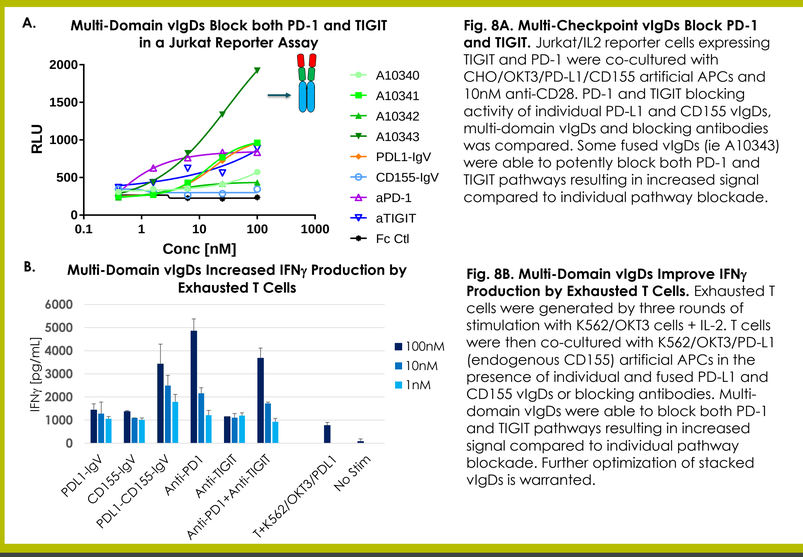


Fig. 8A. Multi-Checkpoint vIgDs Block PD-1 and TIGIT. Jurkat/IL2 reporter cells expressing TIGIT and PD-1 were co-cultured with CHO/OKT3/PD-L1/CD155 artificial APCs and 10nM anti-CD28. PD-1 and TIGIT blocking activity of individual PD-L1 and CD155 vIgDs, multi-domain vIgDs and blocking antibodies was compared. Some fused vIgDs (ie A10343) were able to potently block both PD-1 and TIGIT pathways resulting in increased signal compared to individual pathway blockade.

Fig. 8B. Multi-Domain vIgDs Improve IFNγ Production by Exhausted T Cells. Exhausted T cells were generated by three rounds of stimulation with K562/OKT3 cells + IL-2. T cells were then co-cultured with K562/OKT3/PD-L1 (endogenous CD155) artificial APCs in the presence of individual and fused PD-L1 and CD155 vIgDs or blocking antibodies. Multi-domain vIgDs were able to block both PD-1 and TIGIT pathways resulting in increased signal compared to individual pathway blockade. Further optimization of stacked vIgDs is warranted.

Summary and Conclusions

- Due to vIgD's small size, modular nature, and easily tailored affinities, the vIgD platform is capable of creating multiple novel biologics with potential immuno-oncology applications.
- To demonstrate the versatility of the platform, several unique approaches were tested:
  - A panel of vIgDs were created with unique binding profiles to CD28, CTLA4, and PD-L1. These domains were able to provide a costimulatory signal *in vitro* and confer anti-tumor activity *in vivo*.
  - Costimulatory vIgDs were successfully fused to a HER2-specific antibody and shown to maintain binding and confer costimulatory activity.
  - Individual, high-affinity vIgD domains were combined into multi-domain fusion molecules able to bind and block multiple inhibitory receptors.
- Conclusion: The vIgD platform is both unique and versatile and is poised to contribute to the next generation of immuno-oncology therapeutics. Efforts are ongoing to identify and develop appropriate candidates for clinical trials.