B Cell Modulatory Variant TNF Receptor Domains (vTDs) Identified by Directed Evolution to Inhibit BAFF and APRIL, Alone or Combined with Variant Ig Domains (vIgD™) that Inhibit T Cell Costimulation, for the Treatment of Systemic Lupus Erythematosus and Other Severe Autoimmune Diseases

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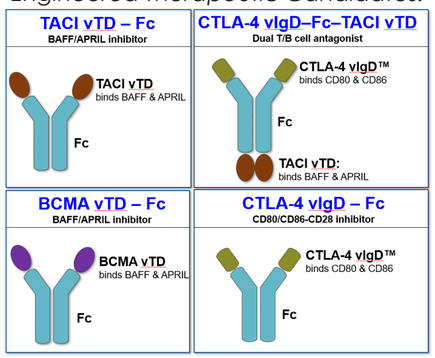
Abstract

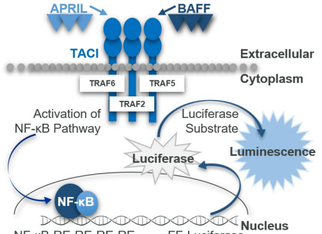
We used our directed evolution platform to identify variant domains of the TNF family receptors TACI or BCMA that exhibit enhanced affinity for BAFF and APRIL as compared to their wild-type (WT) counterparts. These variant TACI or BCMA TNFR domains (vTD), alone or together with a platform-derived CTLA-4 Ig domain (vIgD™), were rendered as Fc fusions, yielding a panel of immunomodulatory molecules: TACI vTD-Fc, BCMA vTD-Fc, TACI vTD/CTLA-4 vIgD-Fc, & BCMA vTD/CTLA-4 vIgD-Fc. All were evaluated for functional activity *in vitro* and *in vivo* vs. comparators like Atacicept and Telitacicept (WT TACI-Fc), and a subset of the data are presented here.

Figure 2: Fc Fusions of Variant TACI, BCMA, and/or CTLA-4 Domains Demonstrate Potent Activity *In Vitro*

Engineered Therapeutic Candidates:



TACI-Jurkat Screening Assay:



IC_{50} Values from In Vitro Functional Assays:

Protein	Human APRIL	Human BAFF	Mouse APRIL	Mouse BAFF	Human CD80	Human CD86
*Belimumab	–	2496	–	1725	–	–
Atacicept	3849	5771	270	1322	–	–
Telitacicept	9103	7699	380	2445	–	–
Abatacept	–	–	–	–	9	240
TACI vTD 1	179	1216	16	255	–	–
TACI vTD 2	262	1387	71	587	–	–
TACI vTD 3	339	1336	30	364	–	–
CTLA-4 vIgD	–	–	–	–	34	8
CTLA-4 vIgD-Fc-TACI vTD	272	1259	26	297	57	10
BCMA WT	183	832	6	673	–	–
BCMA vTD 1	180	1778	6	362	–	–
BCMA vTD 2	190	1532	13	448	–	–
BCMA vTD 3	209	781	6	381	–	–

*Belimumab, Atacicept, and Telitacicept were produced at AIS in HEK-293 cells from published sequences. Abatacept was purchased from Catalent, Inc. BAFF/APRIL blockade was evaluated in the TACI-Jurkat assay described. CD80/CD86 blockade was evaluated with co-culture of Jurkat T cells and artificial K562 APC expressing OKT3 (anti-CD3) and CD80 or CD86.

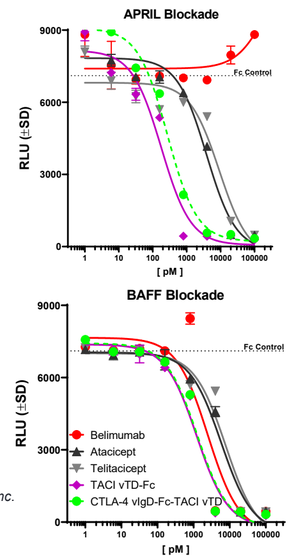


Figure 1: Engineering Optimized Best-in-Class and First-in-Class Therapeutics to Treat Serious Diseases

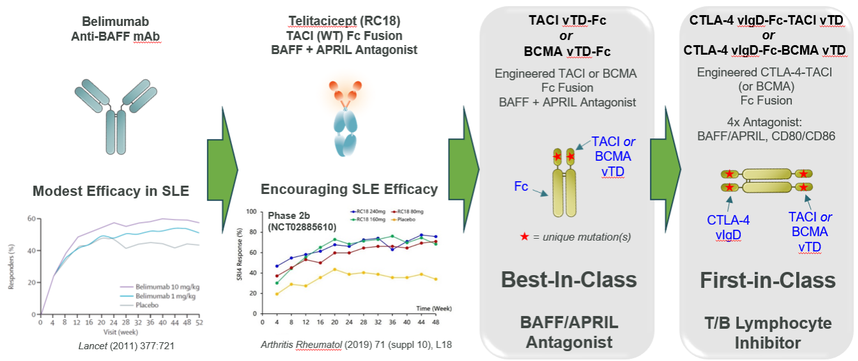


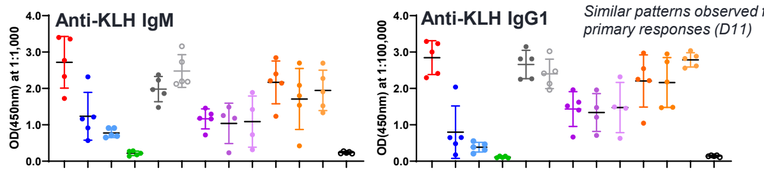
Figure 3: Fc Fusions of Variant TACI, BCMA, and/or CTLA-4 Domains Potently Inhibit T Cell-Dependent Antibody Formation in KLH-Immunized Mice

Female C57BL/6 mice were injected IP with 250 μg endotoxin-free KLH in PBS w/o adjuvant on Days 0 & 12. Test articles were molar matched to 20 mg/kg CTLA-4 vIgD-Fc-TACI vTD 1 (*i.e.*15.5 mg/kg TACI vTD-Fc, etc.) and administered IP on Days 4 & 11 (5 mice/group). Spleens were harvested on Day 20 and analyzed by flow cytometry. Serum was collected 24 hr post each dose for exposure, and for anti-KLH titers on Day 11 & Day 20.

Fc Control, Abatacept, CTLA-4 vIgD-Fc, CTLA4 vIgD-Fc-TACI vTD, Atacicept, Telitacicept, TACI vTD 1-Fc, TACI vTD 2-Fc, TACI vTD 3-Fc, BCMA vTD 1-Fc, BCMA vTD 2-Fc, BCMA vTD 3-Fc, Naive

A. Secondary KLH response (9d post-dose 2; Study Day 20)

Similar patterns observed for primary responses (D11)



B. Enumeration of splenic B cell subsets by flow cytometry (Study Day 20)

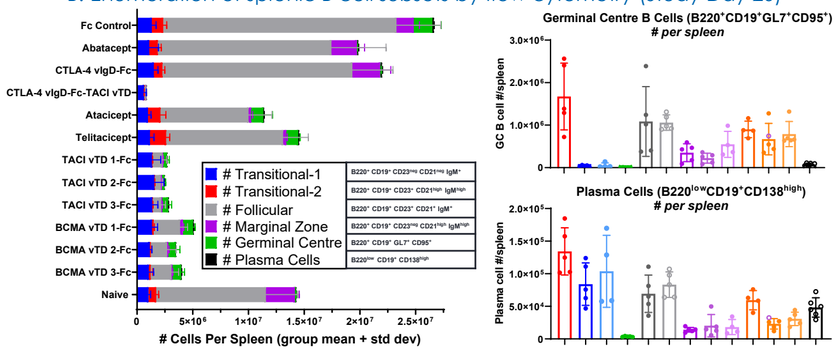
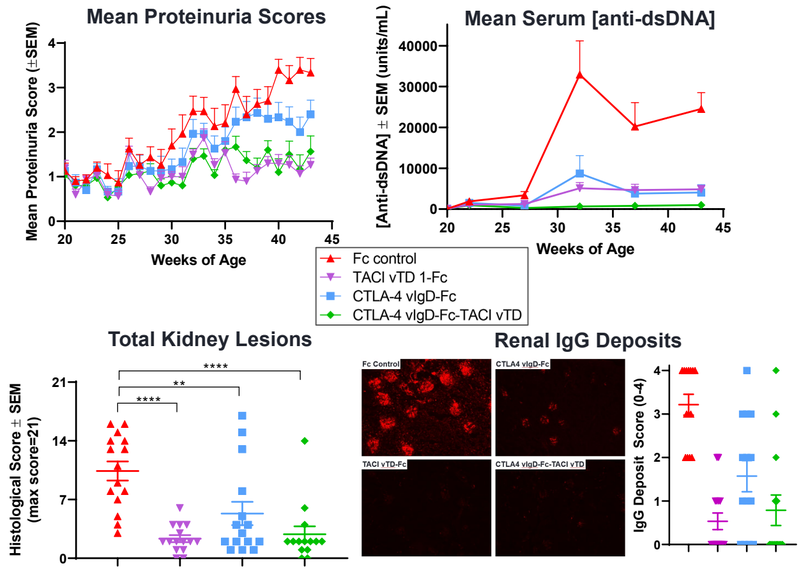


Figure 4: Fc Fusions of Variant TACI and/or CTLA-4 Domains Significantly Suppress Disease in the NZB/W Mouse Model of SLE

Twice weekly treatment of molar matched doses of Fc control (14 mg/kg), TACI vTD 1-Fc (17 mg/kg), CTLA-4 vIgD-Fc (21 mg/kg), or CTLA-4 vIgD-Fc-TACI vTD 1 (25 mg/kg) of (NZBxNZW)F1 mice (15 mice/group) started at 22 wks of age and continued through 43 wks of age. Disease developed as expected in the Fc control group; 11 out of 15 mice developed severe proteinuria and 8 of those mice were euthanized early due to disease severity.



Thanks to Hooke Laboratories (Lawrence, MA) for conducting the NZB/W study. Histology scored as per Alperovich et al. (2007) Lupus 16:18-24. Glomerular IgG deposition scored as per Kelkka et al. (2014) Antioxid Redox Signal. 21:2231-45.

Summary and Conclusions

- Directed evolution of TNFR and IgSF domains has successfully facilitated the development of Fc fusion proteins containing TACI or BCMA vTDs, with or without fusion to CTLA-4 vIgDs.
- These novel proteins consistently demonstrate potent immunomodulatory activity and efficacy *in vitro* and *in vivo*, superior to approved and/or clinical candidate therapeutics like belimumab, abatacept, atacicept, or telitacicept.
- Such novel biologics may therefore be attractive development candidates for the treatment of serious autoimmune and/or inflammatory diseases, particularly B cell-related diseases such as SLE, Sjögren's syndrome, and other connective tissue diseases. Preclinical development to enable clinical trials has been initiated.