Molecular design of CD80 variants with increased affinity for PD-L1 and wild-type or lower affinity for CD28

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SITC 2026 Abstract **#406**

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BACKGROUND

CD80 (B7-1) is a critical ligand for CD28 on T cells, permitting co-stimulation and thereby full effector cell activation. However, safe therapeutic targeting of CD28 is challenging as superagonistic stimulation of CD28 can induce cytokine storm and potentially multi-organ damage. CD80 also interacts with PD-L1 on antigen-presenting or cancer cells to prevent PD-L1:PD-1-mediated deactivation of T cells, and with CTLA-4, a decoy receptor on T cells that diminishes CD28 co-stimulation. Soluble CD80-Fc proteins were developed to mimic such effects. Here, we demonstrate a molecular modeling approach to increase the affinity of CD80 for PD-L1 without increasing the affinity for CD28 to avoid immune overstimulation.

METHODS

Molecular models were built with the human CD80 extracellular domain (ECD) bound to PD-L1, CD28, or CTLA-4 using standard molecular modeling techniques. CD80 mutations predicted to increase the affinity for PD-L1 or reduce the affinity for CD28 were identified using DDMut-PPI (https://biosig.lab.uq.edu.au/ddmut_ppi/) and SSIPe (https://zhanggroup.org/SSIPe/) (**Figure 1**). A subset of these mutations was validated by surface plasmon resonance (SPR) with CD80 ECD-Fc constructs.

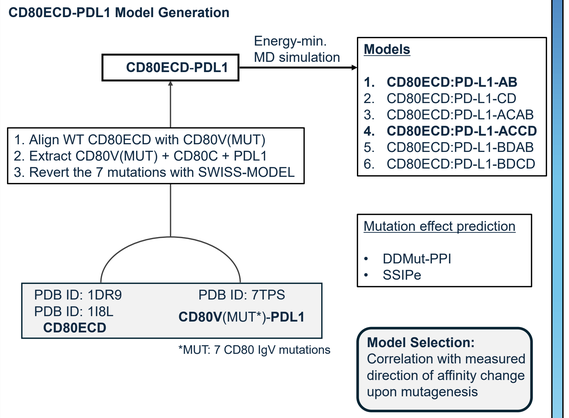


Figure 1. Generation of models with human CD80 extracellular domain (ECD) bound to human PD-L1.

CONCLUSIONS AND OUTLOOK

- **CD80 can be engineered for customized binding affinities for PD-L1 while essentially maintaining wild-type affinity for CD28.**
- **To fine-tune the affinities for PD-L1, CD28, and CTLA-4, additional CD80 mutations are being evaluated.**
- **CD80 mutations are introduced in TILsRx targeted immune checkpoint inhibitors for clinical development (see SITC 2026 abstract/poster # 405).**

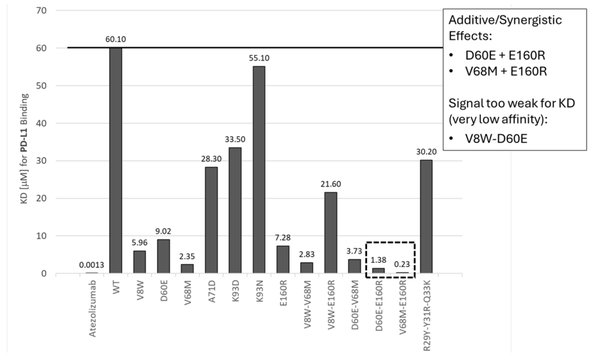


Figure 6. Effects of CD80 mutations in CD80 ECD-Fc constructs on PD-L1 affinity. Affinity was measured by surface plasmon resonance (SPR). Various CD80 mutations were tested in CD80 ECD-Fc constructs for their effects on affinity for PD-L1. The V68M/E160R double mutation (used in TILsRx-001, see SITC 2026 abstract/poster #405) had the strongest effect, enhancing the affinity to 0.23 μM.

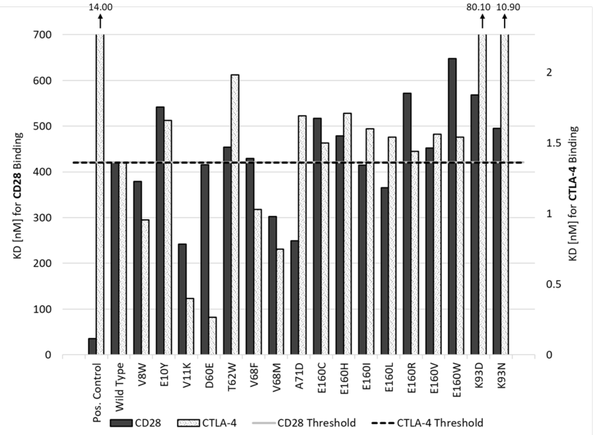


Figure 7. Effects of CD80 mutations in CD80 ECD-Fc constructs on CD28 and CTLA-4 binding. Some mutations increasing CD80:PD-L1 binding potentially also affect CD80:CD28 or CD80:CTLA-4 binding. This is particularly apparent for D60E, which may increase the affinity for CTLA-4 but not for CD28. The horizontal, overlapping, lines indicate the wild-type affinities (KD).

RESULTS

- V8W, V11K, D60E, T61W, V68F, V68M, E160C, E160H, E160I, E160L, E160R, E160V, E160W, V8W/V68M, D60E/E160R, and V68M/E160R mutations increased the affinity for PD-L1 as assessed by SPR. Wild-type (WT) affinity (KD) for PD-L1 was in most SPR experiments > 30 μM. V68M/E160R yielded an affinity of 0.23 μM, representing a > 130-fold increase—the highest among the aforementioned mutations (**Figure 6**).
- D60E both increased the affinity for PD-L1 (from > 30 μM to 4.5-9 μM) and increased the affinity for CTLA-4 five-fold (from 1.36 nM to 0.27 nM), without affecting the affinity for CD28 (KD WT: 421 nM, KD D60E: 416 nM; **Figures 5-7**).

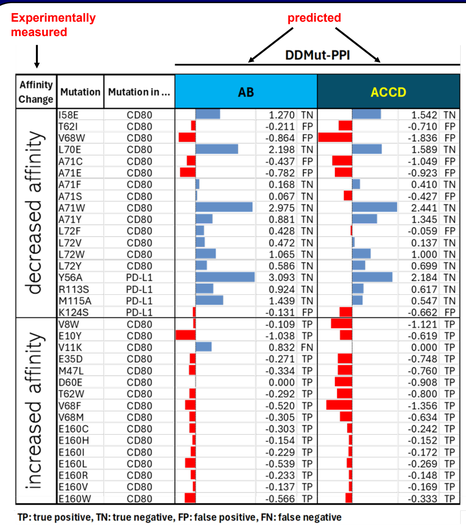


Figure 2. Diagnostic features of models CD80ECD:PD-L1-AB ("AB") and CD80ECD:PD-L1-ACCD ("ACCD"). Predicted effects of CD80 and PD-L1 mutations on CD80:PD-L1 affinity and their correlation with binary experimentally established data (increased vs. decreased affinity).

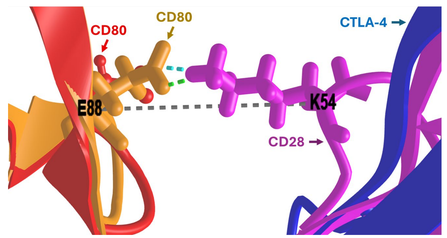


Figure 4. Targeting structural differences between CD28 and CTLA-4 model.

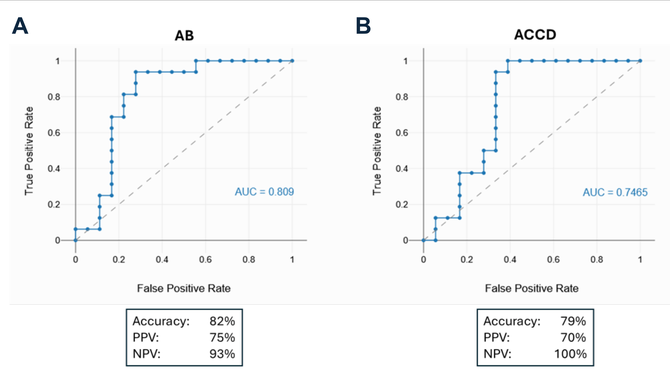


Figure 3. Predictive potential of models CD80ECD:PD-L1-AB ("AB") (A) and CD80ECD:PD-L1-ACCD ("ACCD") (B). Receiver Operating Characteristic (ROC) curves. Using the experimentally validated data set from **Figure 2**, with model AB, an accuracy of 82%, and with model ACCD, of 79% was obtained. PPV, positive predictive value; NPV, negative predictive value.

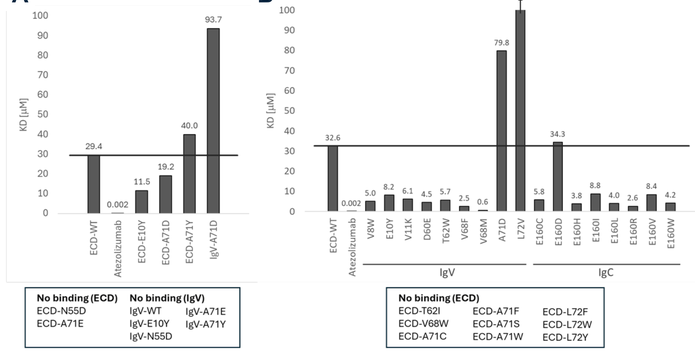


Figure 5. Affinity of single mutant CD80 ECD variants for PD-L1. A. First SPR experiment. **B.** Second SPR experiment.