Directed Evolution of Existing Biologics for Novel Target Specificity, Affinity, and Local Environment

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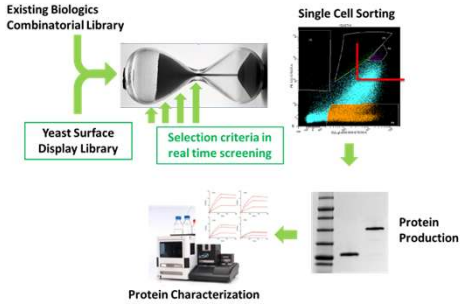
Abstract

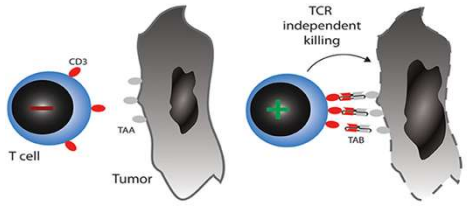
Directed evolution was exploited to optimize existing biologics for target specificity, target-binding affinity and environment-specific binding. The technique has been successfully applied to (1) engineer pH-dependent anti-CD3 antibody for conditional binding in the acidic tumor microenvironment; (2) obtain PD1 variants with high affinity to both PD-L1 and PD-L2 ligands and (3) obtain HVEM variants with high-affinity to both ligands BTLA and CD160 while no binding to another ligand LIGHT

Directed Protein Evolution Platform



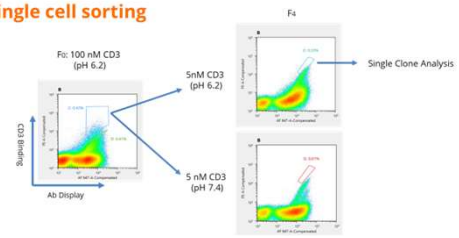
Example 1: Engineering pH-dependent CD3 antibody

Anti-CD3 antibody is used in T-cell activation bispecific antibodies (TAB). Presence of tumor-associated antigen in normal tissues may cause the on-target off-tumor toxicity, limiting the application of TAB for solid tumors. We engineered a pH-dependent anti-CD3 antibody for conditional binding in the acidic tumor microenvironment

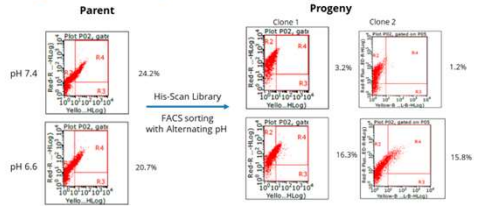


Kobold et al., 2018

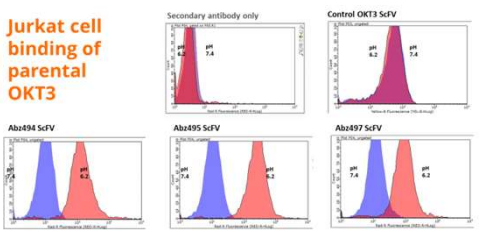
Single cell sorting



Single clone analysis

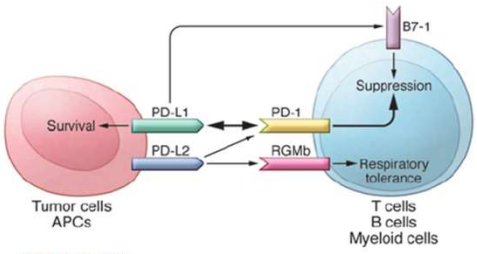


Jurkat cell binding of parental OKT3



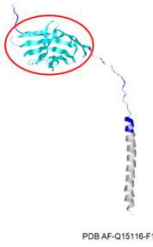
Example 2: PD1-based PD-L1/PD-L2 dual blocker

Blocking PD-1 signaling has revolutionized the field of cancer immunotherapy, however, most patients do not respond to this approach. The PD pathway has at least 5 interacting molecules. PD-L1 and PD-L2, identified as ligands of PD-1, and the interaction of PD-L1 or PD-L2 with PD-1 may induce T cell suppression. PD-L1 was found to interact with B7-1 (CD80) on activated T cells and inhibit T cell activity. PD-L2 has a second receptor, RGMb; initially, this interaction activates T cells, but it subsequently induces respiratory tolerance. PD-L1 on tumor cells can also act as a receptor, and the signal delivered from PD-1 on T cells can protect tumor cells from cytotoxic lysis. Blocking both PD-L1 and PD-L2 will offer a more comprehensive checkpoint blockade compared to blocking PD-1 alone.



L. Chen & X. Han, 2015

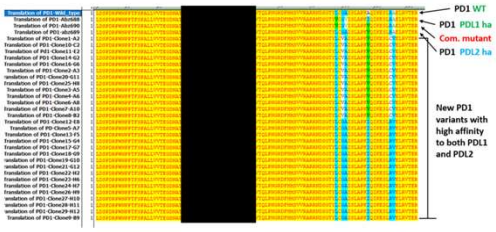
Since PD-L1 and PD-L2 share low homology, it would be difficult to develop a monoclonal antibody reactive with both PD-L1 and PD-L2. PD1 receptor binds to both ligands, but with low affinity. We engineered soluble PD1 variants that bind to both PD-L1 and PD-L2 with high affinity.



Uniprot Q15116 Extracellular domain AA24-170

Goal: Generate PD-1 variants to bind both human and mouse PD-L1 and PD-L2 with High Affinity

Using directed evolution, we have identified two PD1 receptor variants that bind to both PD-L1 and PD-L2 with high affinity



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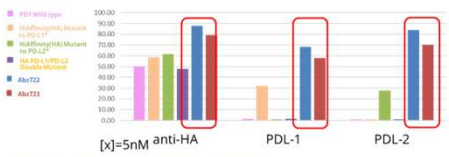
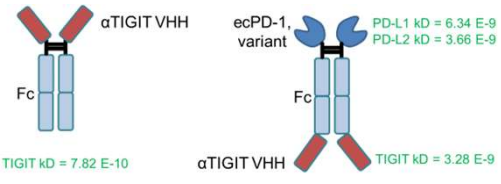
Displayed on cell surface, Abzyme's PD1 variants have high affinity to both PDL1 & PDL2 while published Mutant 1* and Mutant 2* have high affinity only to either PDL1 or PDL2.



*HiAffinity PD-L1: Maecker et al., Stanford/NIH, Harvard Medical School, PNAS, 2015
*HiAffinity PD-L2: Tang & Kim, Stanford ChEM-H, PNAS, 2019.

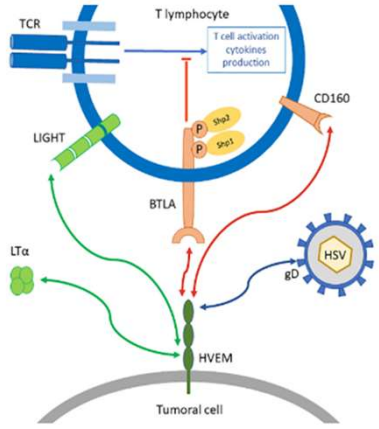
Modular PD1 Variant as a Component of Bispecific Therapeutics



Example 3: HVEM-based CD160/BTLA specific blocker

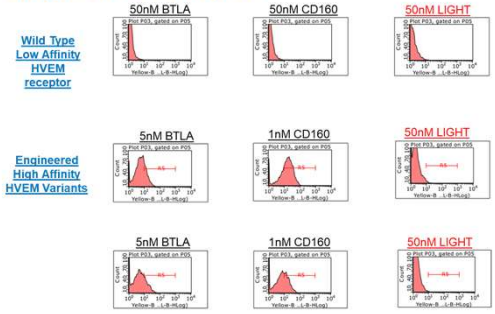
Multiple receptors and ligands are involved in the immune checkpoint regulation. Among them are HVEM receptor and its ligands CD160, BTLA and LIGHT. BTLA and CD160 are inhibitory ligands, while LIGHT is a costimulatory ligand. Anti-BTLA antibodies (Icatolimab, Tifcemalimab) in monotherapy and in combination with anti-PD-1 antibody are in clinical trials for treatment of advanced solid tumors. Anti-CD160 antibodies are being explored for treatment if various human diseases including cancer and retinal neovascular diseases. Anti-CD160 has the potential to 1) block the checkpoint inhibition, 2) reverse the T cell anti-tumor immune function, 3) stimulate IFNγ production, 4) reactivate the NK cell-mediated anti-tumor immune responses and 5) block tumor vascularization. In addition, as CD160 expression is found in 98% of chronic lymphocytic leukemias; 100% of hairy cell leukemias and 15% of mantle cell lymphoma patients, anti-CD160 can be used to recognize the CD160 expressed in B cell malignancies and to trigger antibody-dependent cell cytotoxicity.

We engineered HVEM receptor into soluble variants with high affinity to tow inhibitory ligands two BTLA and CD160, but with insignificant binding to LIGHT.

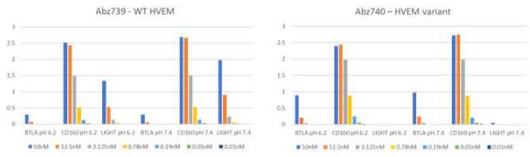


Demerle et al., 2021

Single clone analysis



Soluble HVEM variant has improved BTLA and CD160 binding but not LIGHT



Where Abzyme Can Help