Cytokine affinity tuning using the *AlphaSeq* platform to generate targeted immuno-oncology therapeutics

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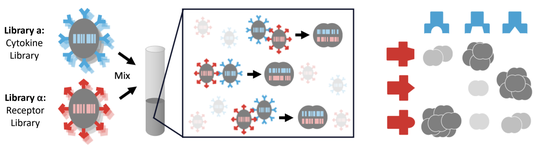
Abstract

Systemic cytokine therapies have been associated with clinical toxicities, creating a narrow therapeutic index. One solution is to engineer detuned cytokine variants that enable antibody mediated cell-specific signaling. Here we apply the *AlphaSeq* platform, which enables library-on-library screening of protein interactions, to measure large interactions networks of human, cynomolgus, mouse, rat, and mutant cytokines and their associated receptors. From this approach, we have identified therapeutic candidates with a wide spectrum of affinities and signaling potencies.

AlphaSeq Technology

The ***AlphaSeq*** platform uses a modified yeast surface display system and a next generation sequencing readout to quantitatively measure millions of protein-protein interactions at a library-on-library scale.

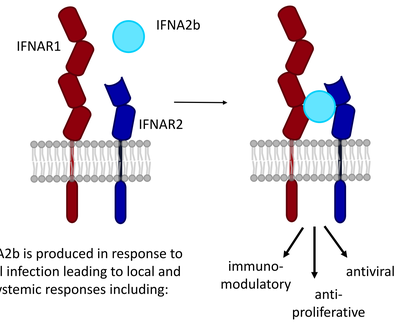


Receptor and ligand yeast display libraries are built and mixed

Interactions between receptors and ligands drive cellular fusion

Cellular fusions are counted with NGS, giving a quantitative readout

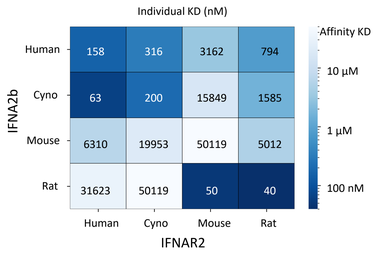
IFNA2b: approved immuno-oncology therapy limited by toxicity



IFNA2b is produced in response to viral infection leading to local and systemic responses including:

IFNA2b is a Type I Interferon, signaling through the IFNAR1 and IFNAR2 complex. Recombinant IFNA2b therapeutics provide clinical benefit in the oncology and infectious disease setting. To reduce systemic IFNA2b signaling, we aim to detune the affinity of IFNA2b toward the high affinity IFNAR2 chain and specifically localize the detuned variant via cell-type specific antibodies.

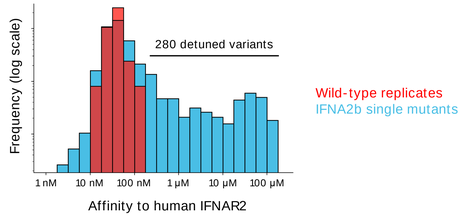
IFNA2b / IFNAR2 binding is validated in *AlphaSeq*



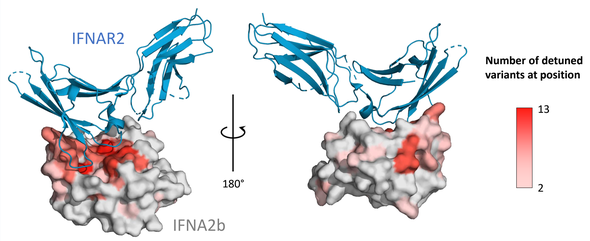
Human IFNA2b binding in *AlphaSeq* was observed against human, cyno and rat IFNAR2. Cyno IFNA2b binding was observed for human and cyno IFNAR2. Rat IFNA2b binding was observed against mouse and rat IFNAR2. No mouse IFNA2b binding was observed.

AlphaSeq detuning of human IFNA2b and IFNAR2

A site-saturation mutagenesis library of all IFNA2b single mutants was constructed and *AlphaSeq* was used to measure the affinity of each variant to human, cyno, rat, and mouse IFNAR2 simultaneously in a single assay.



280 detuned IFNA2b variants spanning 3 orders of magnitude of affinity to human IFNAR2 were identified.

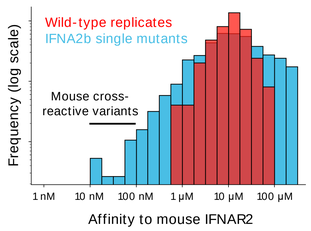


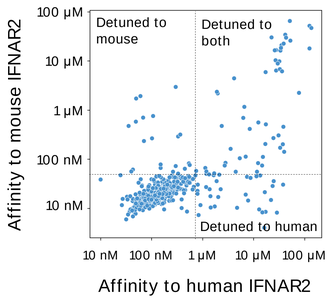
Many IFNA2b detuning mutations were identified near the IFNAR2 binding interface. However, additional mutations distal to the binding interface also led to decreased affinity measurements with *AlphaSeq*.

AlphaSeq parallel detuning of human IFNA2b and mouse IFNAR2

With the goal of moving quickly into mouse syngeneic tumor modeling, *AlphaSeq* detuning against the human and mouse cytokine receptor allows early understanding of candidate species cross-reactivity and enables early surrogate generation, if needed.

Wild-type human IFNA2b binds weakly to mouse IFNAR2 (~10 µM), but individual point mutants confer mouse cross-reactivity to IFNA2b.

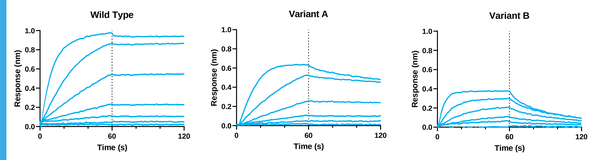


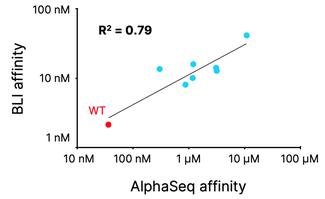


The discovery of mouse cross-reactive IFNA2b variants enabled the design of a second iteration SSM library using one such cross-reactive mutation. Using this library, IFNA2b variants with detuned affinity to both mouse and human IFNAR2 were identified.

BLI affinity measurement of variant IFNA2b Fc fusion proteins correlates with *AlphaSeq* determined KD

Fc-cytokine variants were expressed transiently in mammalian ExpiCHO™ cells and purified by Protein A chromatography. Biolayer Interferometry (Gator Bio) was used to determine binding affinity (KD) of Fc-cytokine variants to immobilized IFNAR2.

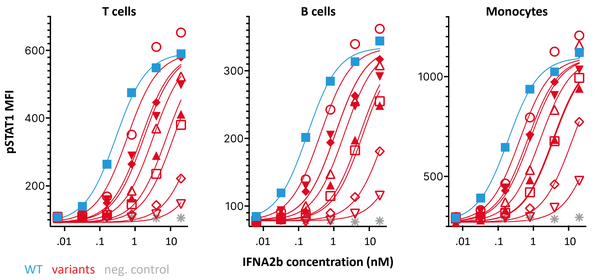




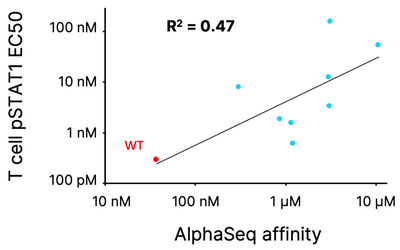
BLI and *AlphaSeq* measured affinities show a strong correlation

AlphaSeq detuned IFNA2b Fc fusion proteins exhibit a range of signaling potency in human PBMC

Human PBMC were stimulated with titrated wild-type IFNA2b Fc fusion protein and detuned variant derivatives for 30 minutes before fixing and permeabilizing cells. Cells were stained with T cell, B cell and monocyte lineage marker antibodies and anti-pSTAT1 MFI determined via flow cytometry.



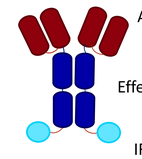
IFNA2b variants detuned with *AlphaSeq* elicit pSTAT1 signaling in human T cells, B cells and monocytes with different kinetics and intensities.

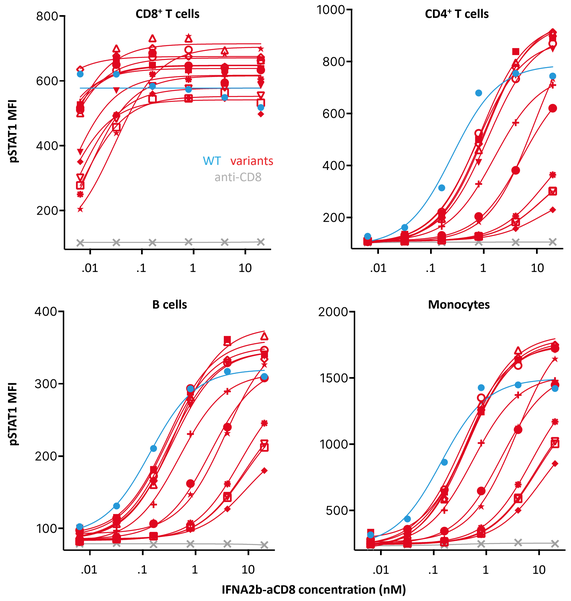


AlphaSeq measured IFNA2b affinity for IFNAR2 correlated with signaling potency determined in human T cells, indicating *AlphaSeq* provides a rapid, therapeutic detuned signaling platform.

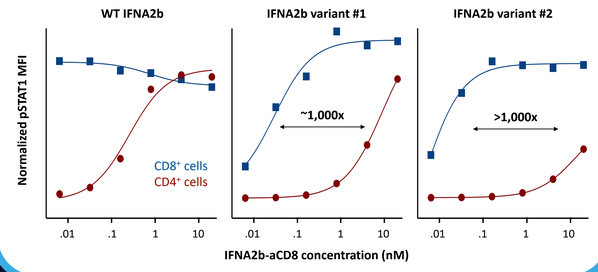
Cell-specific signaling achieved with immunocytokine engineering

IFNA2b variants of interest were fused to anti-human CD8 in a scFv-Fc format, creating an immunocytokine with predicted cis-dependent signaling in CD8 cells. Immunocytokine potency was assayed in human PBMC.





Immunocytokines demonstrated 1,000-fold or greater potency increases in cis-targeted CD8 cells vs CD4 cells in human PBMC cultures.



Conclusions

We demonstrate how *AlphaSeq* can be applied to identify hundreds of detuned cytokines with a spectrum of affinities for targeted receptors. In parallel, variant cross-reactivity for preclinical species of interest can be determined. Exemplary human IFNA2b variants were successfully expressed as Fc fusion proteins whose affinity for recombinant IFNAR2 and signaling in human primary immune cells correlated with *AlphaSeq* determined affinities. Low affinity variants were successfully fused to cellular targeting antibodies for cis-dependent pharmacodynamic activity specific for the desired cell population. We are applying the *AlphaSeq* platform to engineer a portfolio of immunocytokine therapeutics.