Johns Hopkins Engineering

Immunoengineering

Immunoengineering—Allergy and Autoimmunity

Protein Engineering

JOHNS HOPKINS
WHITING SCHOOL
of ENGINEERING

General Outline

- Engineered Cells
- Engineered Microbes
- **Engineered Proteins**
- **Engineered Genetic Material**

Cytokine Engineering Therapies

- Eli Lilly - $400 million
  - Nektar Therapeutics
- Celgene - $300 million
  - Delinia

Ledford, Heidi “Drug companies flock to supercharged T-cells in fight against autoimmune disease Researchers target suppressive cells to keep the body from attacking itself.” *Nature News.* 02 August 2017.

IL-2 Receptor Biasing – Nektar Therapeutics

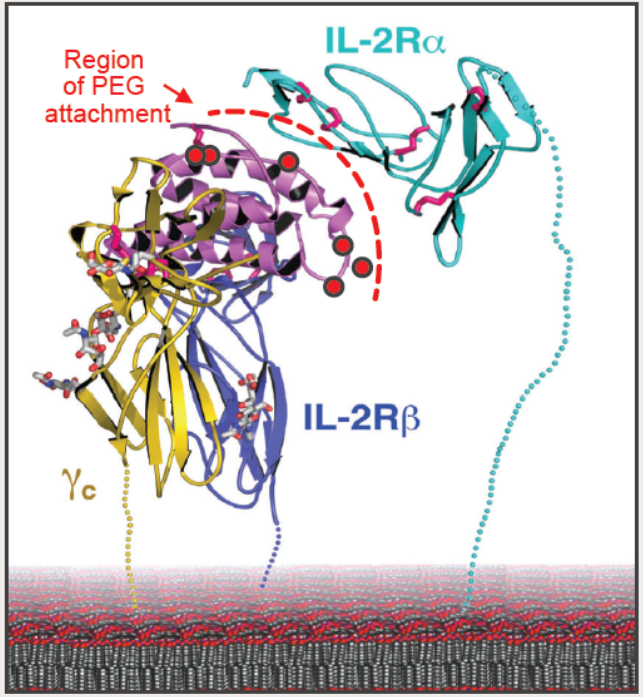
Cell type	CD25 (IL-2Rα)	CD122 (IL-2Rβ)	CD132 (IL-2Rγ)
Naïve T cell	-	- / +	+
Effector T cell	+ + +	+ +	+
Memory T cell	-	+ / + +	+
NK cell	-	+ +	+
Treg cell	+ + +	+	+
Endothelial cell	+	+	+

Adapted from Boyman and Sprent, *Nat Rev Immunol* 12(3):180-90 (2012)

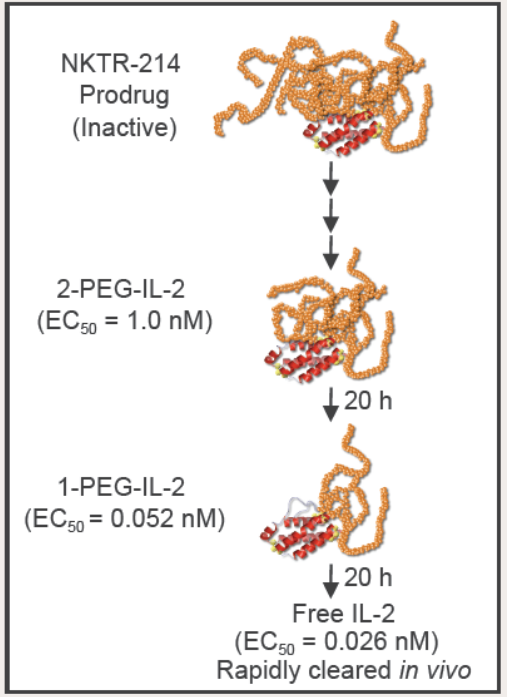
2015 Inaugural CRI-CIMT-EATI-AACR Immunotherapy Conference
Poster: Antitumor activity of NKTR-214, a CD122-biased immunostimulatory cytokine, combined with immune checkpoint blockade requires innate and adaptive immunity
John L. Langowski et al.

IL-2 Receptor Biasing – Attachment of Polymers

- PEG – polyethylene glycol
  - Increase half-life
  - Improve biodistribution
  - Controlled release of active version of the drug
- Specific sites of attachment for PEG prevent binding with specific receptors



Adapted from Wang et al., *Science* Vol 310 18 Nov. 2005



EC_{50}, pSTAT5 CTLL-2

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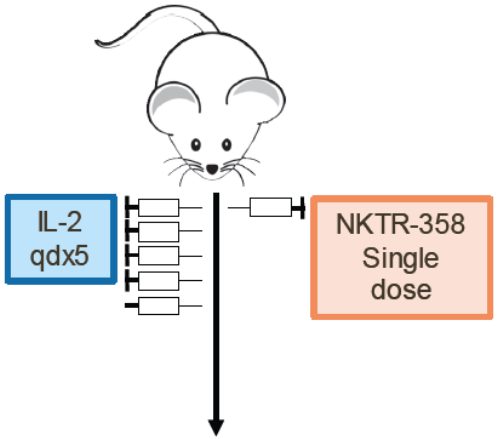
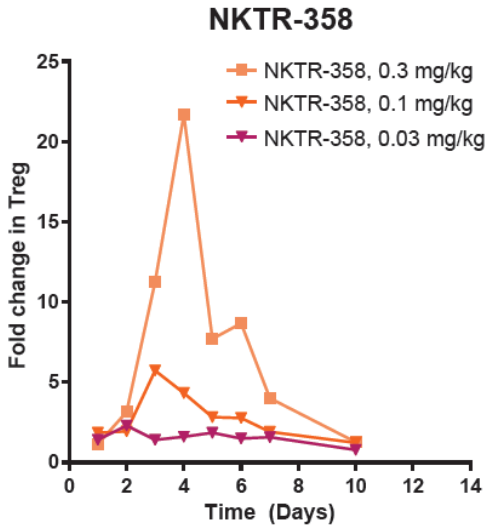
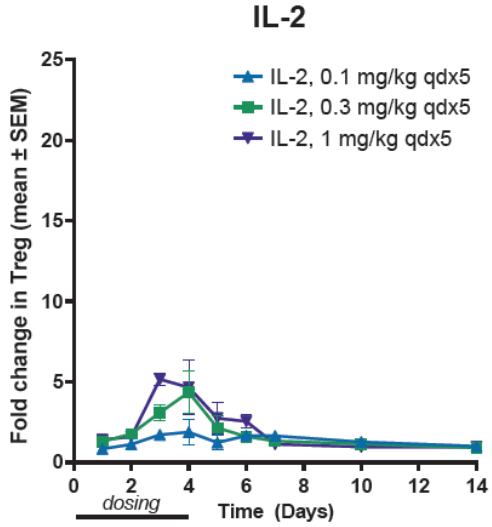
Treg Targeting IL-2

A single administration of NKTR-358 promotes greater Treg mobilization than multiple IL-2 administrations



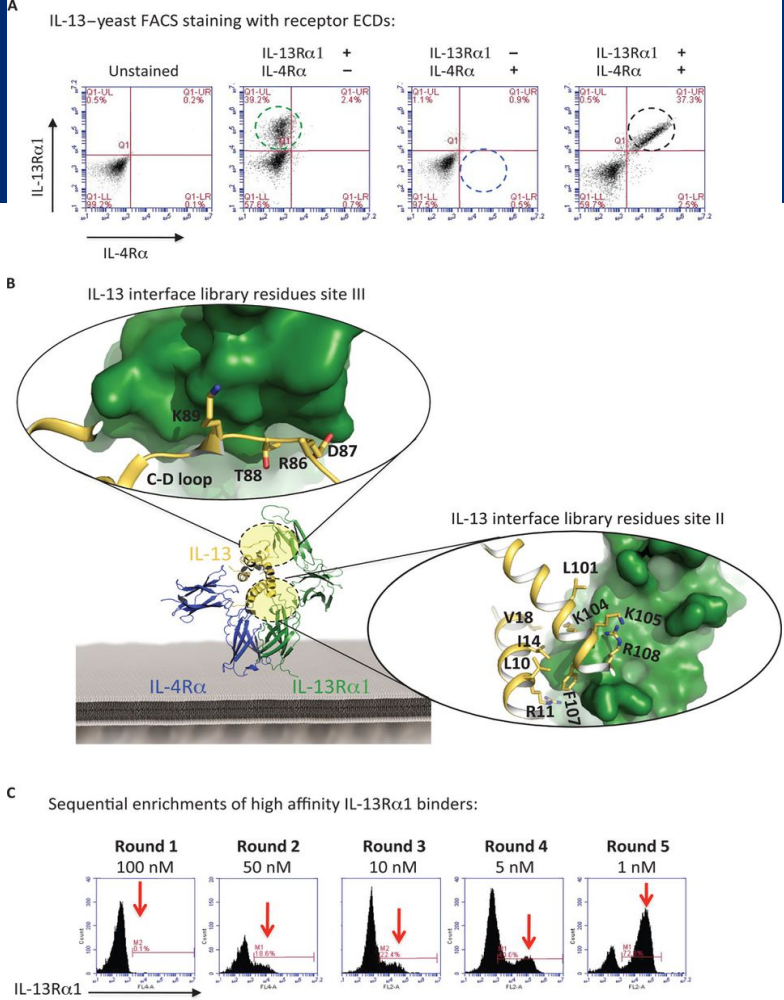




American College of Rheumatology Annual Meeting, San Diego, CA13th Annual World Congress on Inflammation
Poster Abstract 2715: NKTR-358: A Selective, First-in-Class IL-2 Pathway Agonist Which Increases Number and Suppressive Function of Regulatory T Cells for the Treatment of Immune Inflammatory Disorders, Langowski, J., et al.

Cytokine Engineering – IL-13

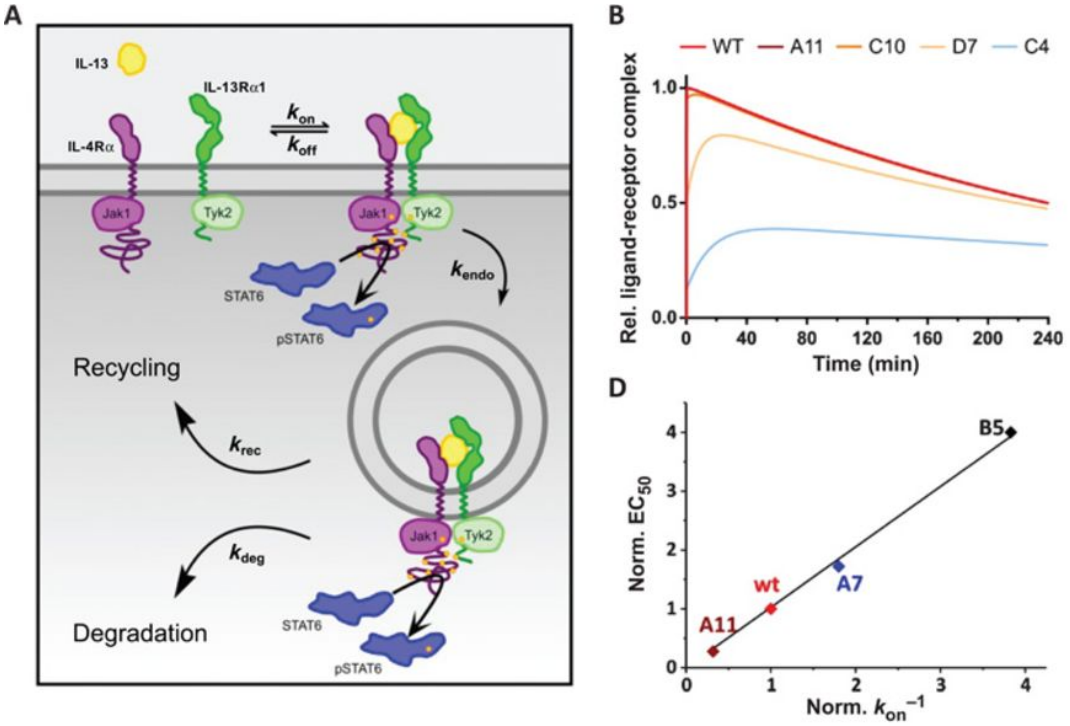
- IL-13 associated with asthma and fibrosis
- Yeast display to engineer variants of binding affinity
- Differential k.on and k.offs



Moraga, Ignacio, et al. "Instructive roles for cytokine-receptor binding parameters in determining signaling and functional potency." *Sci. Signal.* 8.402 (2015): ra114-ra114.

Cytokine Engineering – IL-13 modeling

- Single molecule imaging allowed them to hypothesize a model
- Kinetic model agrees with observed experimental results
  ⬆k.on = ⬆pSTAT forming more complexes
  ⬆k.off = ⬇ STAT activation through endocytosis
  k.e important regulator of low k.off
- Help cytokine therapies to engineer low affinity therapies with equal potency to limit non-specific effects



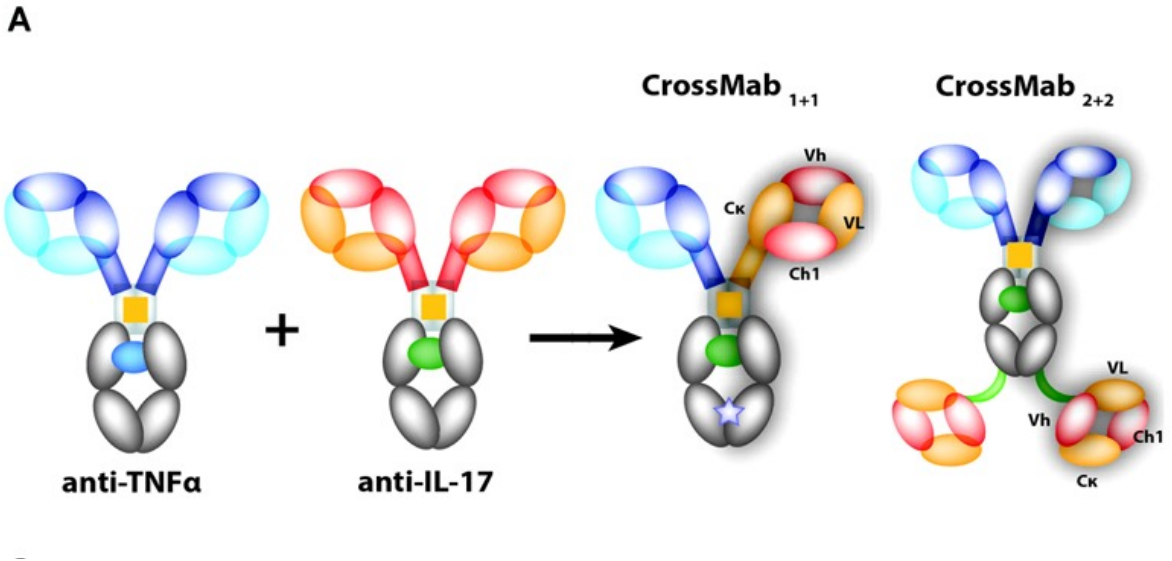
Moraga, Ignacio, et al. "Instructive roles for cytokine-receptor binding parameters in determining signaling and functional potency." *Sci. Signal.* 8.402 (2015): ra114-ra114.

Antibody Engineering – bispecific for RA

- TNFa and IL-17 raised in Rheumatoid arthritis

<u>Treatment effects</u>

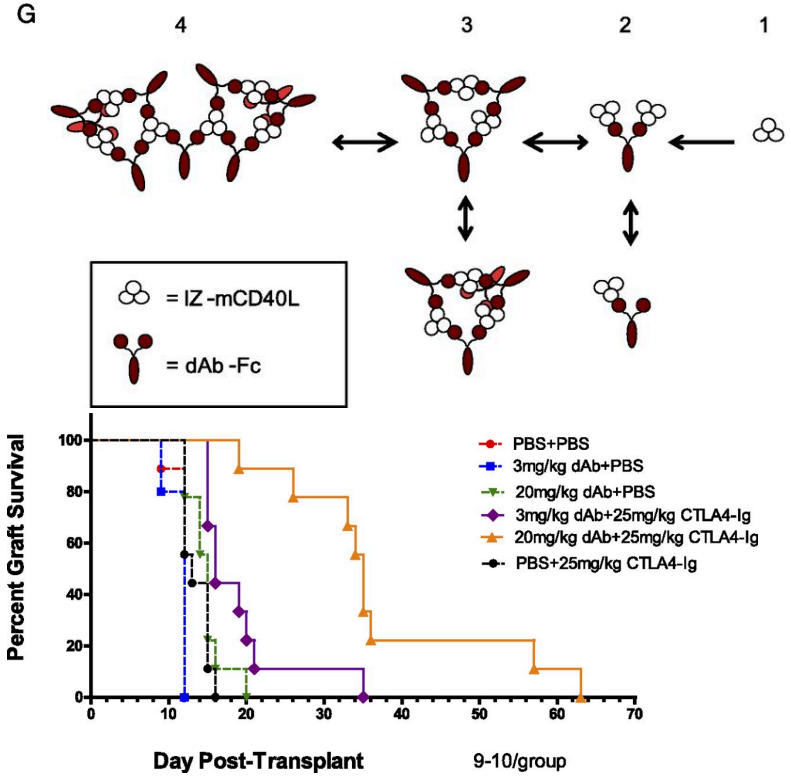
- Decreased levels of cytokines
- Decreased swelling in bone
- 2+2 format more effective than 1+1 format potentially due to higher avidity



Fischer, Jens AA, et al. "Combined Inhibition of Tumor Necrosis Factor α and Interleukin-17 As a Therapeutic Opportunity in Rheumatoid Arthritis: Development and Characterization of a Novel Bispecific Antibody." *Arthritis & Rheumatology* 67.1 (2015): 51-62.

Antibody Engineering – anti-CD40L

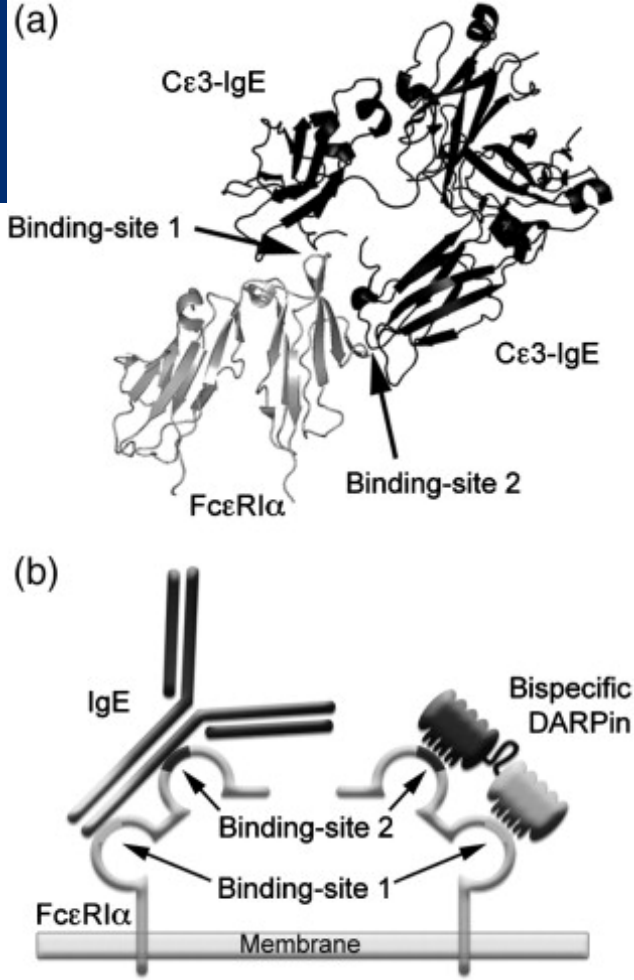
- Blocking CD40L attractive for treating autoimmunity or transplant recipients
  - But cause thromboembolism
- Domain Antibody (dAb)
  - Smallest Ab fragments (10% of size)
  - Eliminate Fc region
  - Mutate and add back non-active Fc region
- Decrease platelet activation while retaining bioactivity



Xie, Jenny H., et al. "Engineering of a novel anti-CD40L domain antibody for treatment of autoimmune diseases." *The Journal of Immunology* 192.9 (2014): 4083-4092.

DARPin for Allergies

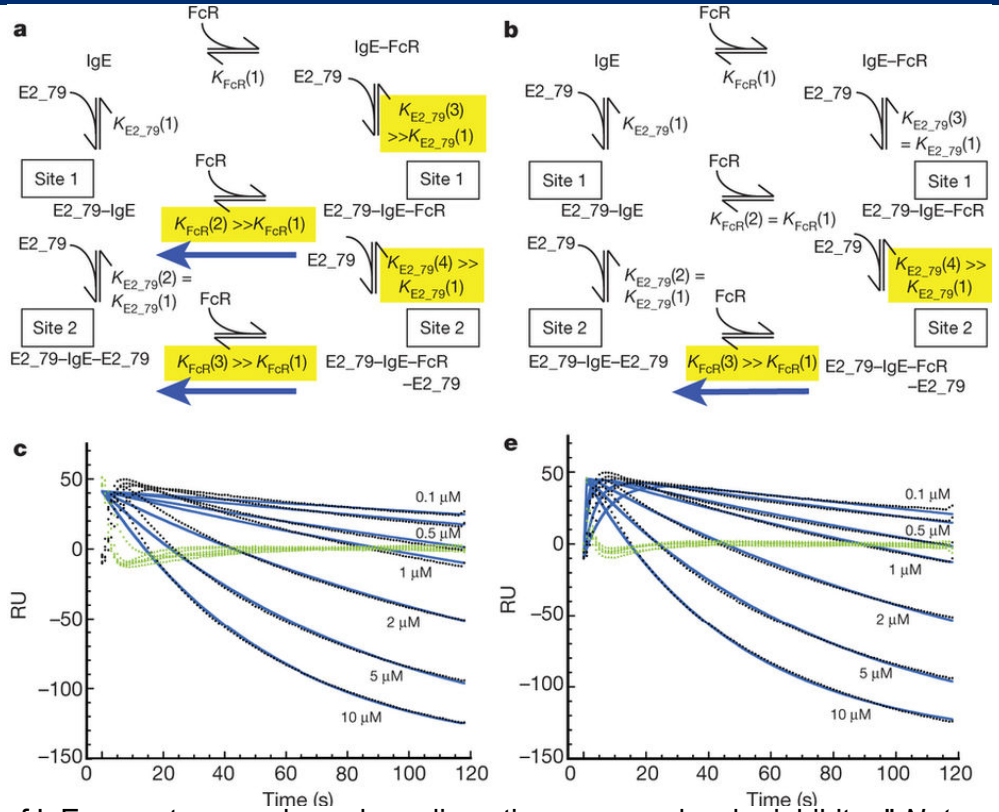
- Antibodies are unable to compete off established IgE/Fce
  - Also bispecific nature leads to crosslinking and further activation
- DARPin (designed ankyrin repeat proteins)
  - Engineered antibody fragments often aggregate
  - Produce bacterially
  - Bi-specific DARPin – target 2 regions on same protein



Eggel, Alexander, et al. "DARPins as bispecific receptor antagonists analyzed for immunoglobulin E receptor blockage." *Journal of molecular biology* 393.3 (2009): 598-607.

DARPin for Allergies

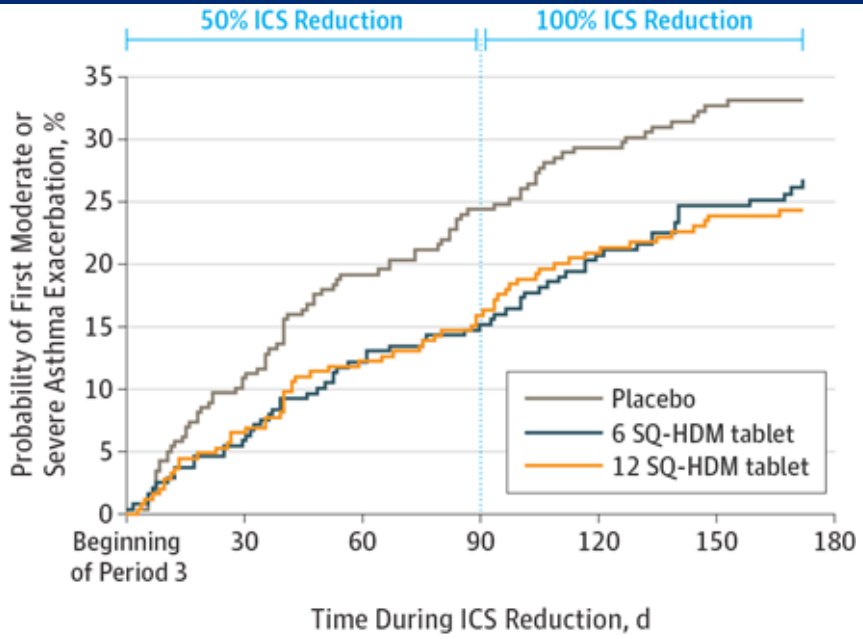
- Kinetic models establish mechanistic facilitated dissociation
- Works in both in vitro and in vivo assays more efficiently than Omalizumab (current antibody therapy)



Kim, Beomkyu, et al. "Accelerated disassembly of IgE–receptor complexes by a disruptive macromolecular inhibitor." *Nature* 491.7425 (2012): 613.

Allergen Peptide Immunotherapy

- One of most common allergy treatments
- Low levels of peptide given over time
- Effective in reducing allergic rhinitis
- Administration with shots or sublingual tablets

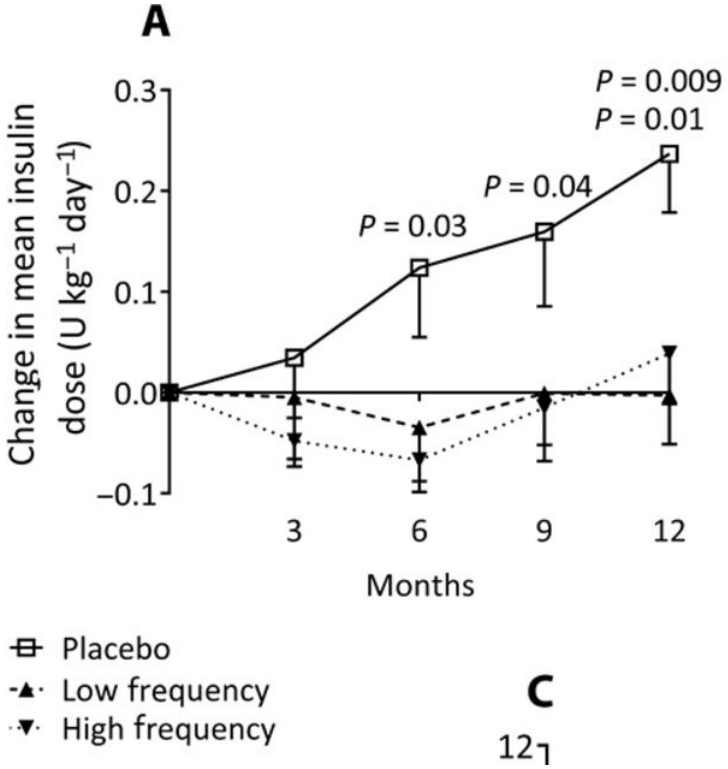


No. at risk							
Placebo	257	228	200	188	171	163	109
6 SQ-HDM tablet	237	224	207	201	187	171	122
12 SQ-HDM tablet	248	228	214	207	189	180	121

Virchow, J. Christian, et al. "Efficacy of a house dust mite sublingual allergen immunotherapy tablet in adults with allergic asthma: a randomized clinical trial." *Jama* 315.16 (2016): 1715-1725.

Short Peptide Immunotherapy for Type 1 Diabetes

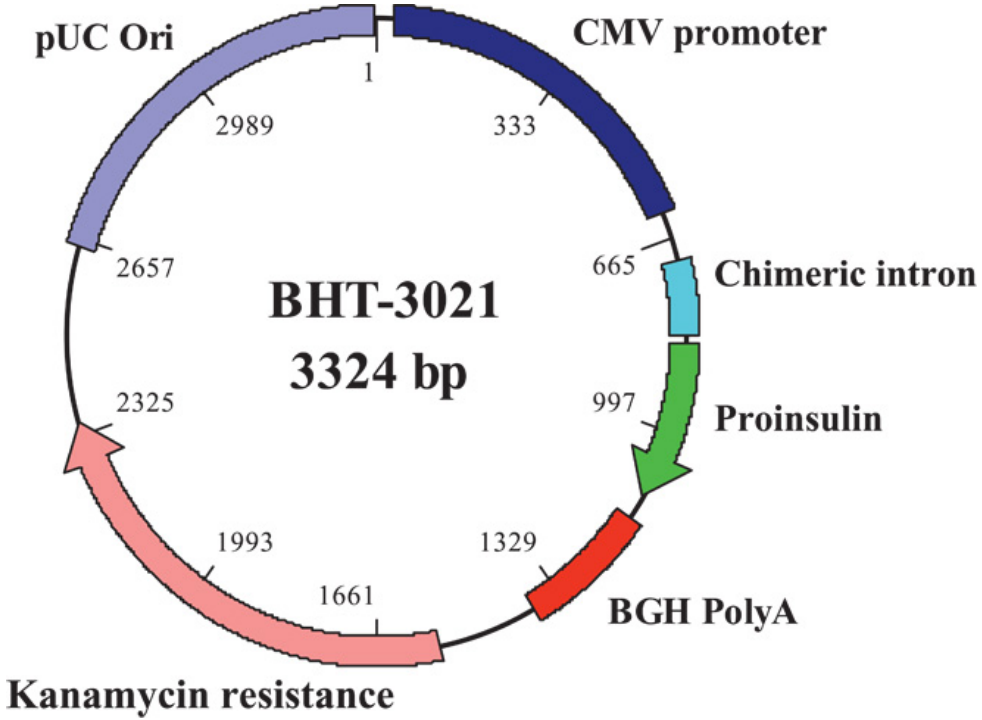
- More risky for autoimmune diseases
- Phase 1 trial
- Pro-insulin peptide given to T1D patients not need to increase insulin



Ali, Mohammad Alhadj, et al. "Metabolic and immune effects of immunotherapy with proinsulin peptide in human new-onset type 1 diabetes." *Science translational medicine* 9.402 (2017): eaaf7779.

DNA Vaccine for Insulin Tolerization

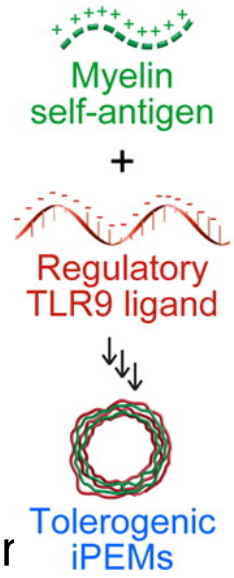
- Similar Phase 1 trial except with DNA vaccine
- Design pro-insulin to be tolerogenic
- Advantages to DNA delivery
  - Ease of manufacturing
  - More cost effective
  - Durability of expression
  - Produce whole protein

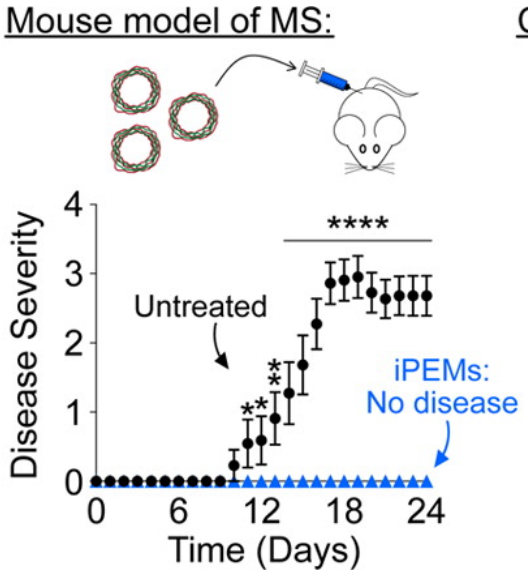


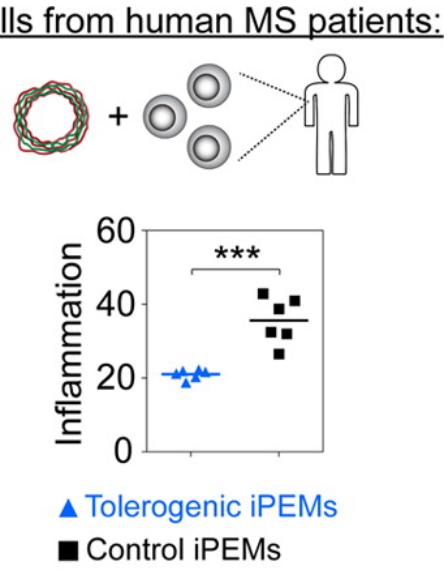
Roep, Bart O., et al. "Plasmid-encoded proinsulin preserves C-peptide while specifically reducing proinsulin-specific CD8+ T cells in type 1 diabetes." *Science translational medicine* 5.191 (2013): 191ra82-191ra82.

Polyelectrolyte Multilayered Immune Modulation

- Bias response with regulatory signals
- Functions of nano-biomaterials without inherent biomaterial inflammation or clearance
- Effective in treating MS mouse models and decreasing inflammatior from human samples



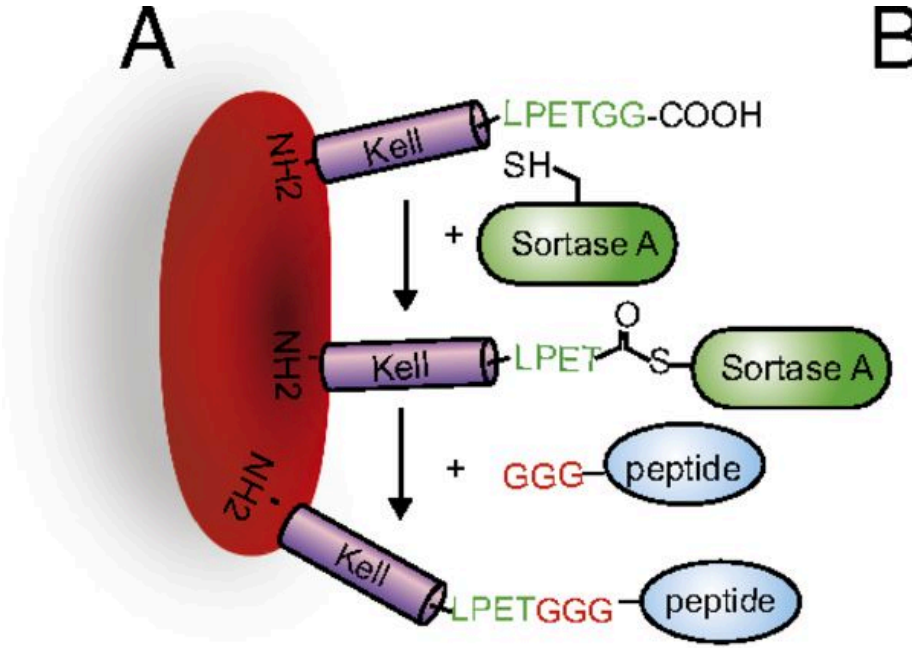




Tostanoski, Lisa H., et al. "Design of polyelectrolyte multilayers to promote immunological tolerance." *ACS nano* 10.10 (2016): 9334-9345.

Combination Therapies – Cell & Peptide Engineering

- Apoptotic cells are tolerogenic
- RBCs are an attractive vehicle
  - RBCs have quick turnover
  - RBCs are abundant and accessible
  - Dead RBCs are cleared by the spleen (secondary lymphoid organ)
- Enzyme covalent modification with autoimmune peptides
- Therapeutic in mouse models of T1D and MS



Pishesha, Novalia, et al. "Engineered erythrocytes covalently linked to antigenic peptides can protect against autoimmune disease." *Proceedings of the National Academy of Sciences* (2017): 201701746.

Summary

- Promising Future for Protein Engineering
  - Improve pharmacokinetics (e.g. half-life)
  - Decrease off-target effects (e.g. specificity)
  - Increased tools (e.g. evolution & modeling)
  - Curative potential (e.g. antigen immunotherapy)
  - Increased understanding (e.g. Fc receptor K.O.)

- Challenges Ahead for Protein Engineering
  - Immunogenicity
  - Safety
  - Cost