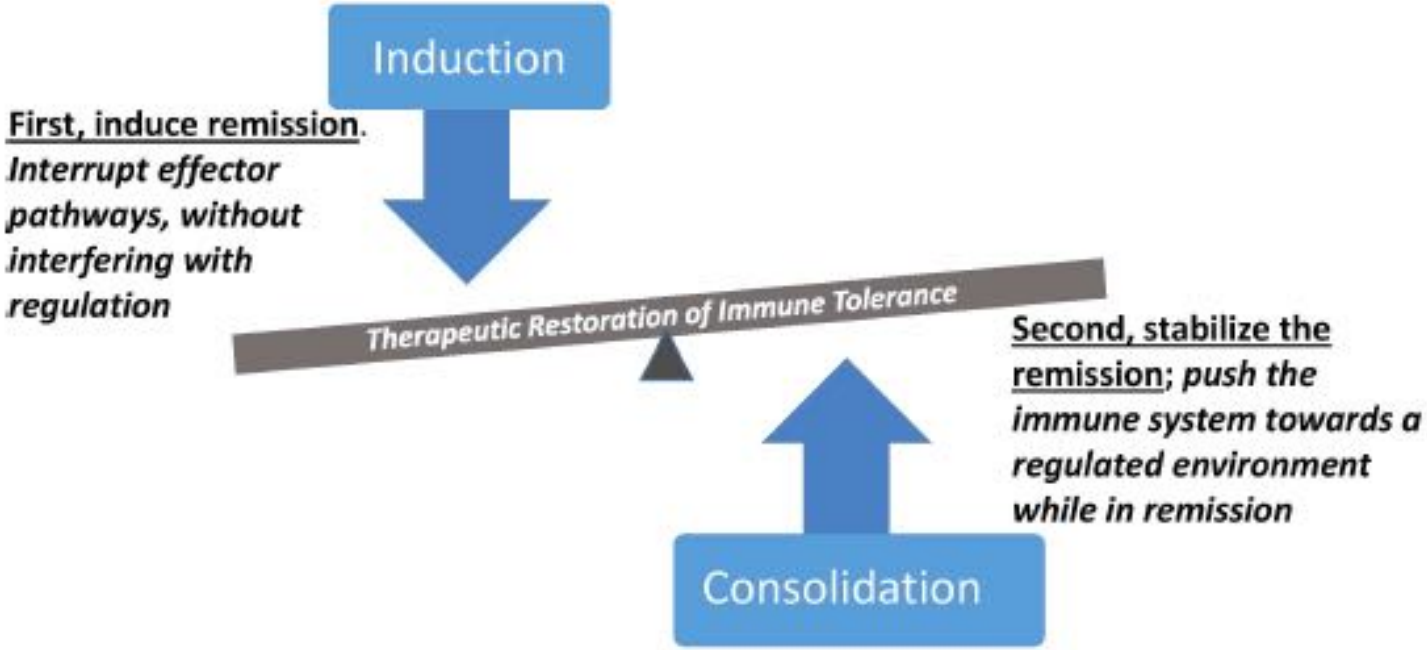
Novel Approaches for Treatment of Autoimmunity: Application to Mitigate Immunogenicity of Biological Therapeutics?

Amy S. Rosenberg, M.D., Senior Director,
Immunology and Consultant, EpiVax, Inc

Setting the Stage for Success in Immune Tolerance Induction in Autoimmunity, Allergy, and Transplantation

EpiVax



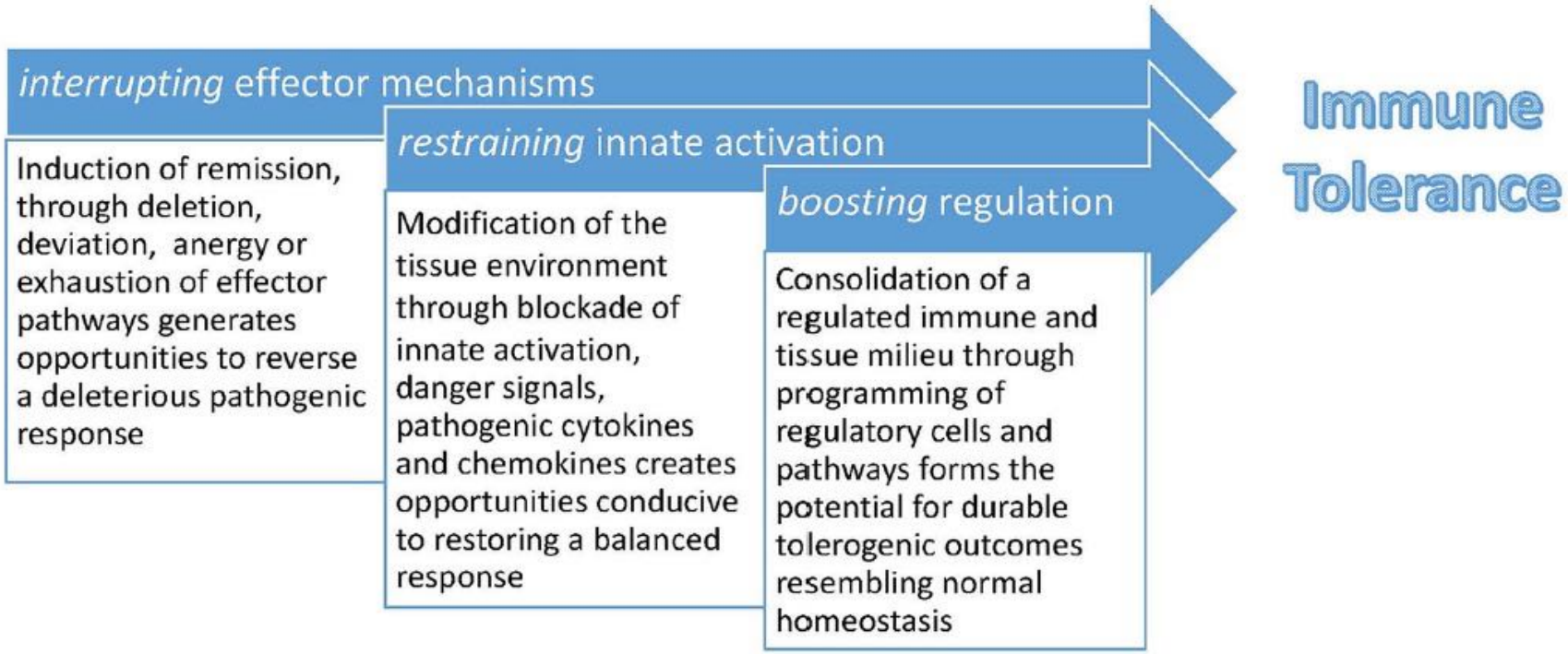
Nepom GT (2022) .Front. Immunol. 13:962177.

Agenda

- Strategic staged approach for immune tolerance induction: focus on autoimmune diseases
- From autoantibodies to autoimmune disease: the role of epitope spread, potential means to stop it and applicability to immunogenicity of biological therapeutics
- Clinical studies for preventing Type 1 Diabetes Mellitus in those at high risk: potential approaches for boosting and activating Tregulatory cells
- Novel modalities for treatment of autoimmunity: application to immunogenicity of biological therapeutics.
  - CD19 CAR-T cells
  - Therapeutic Drug Monitoring (TDM) of TNF mAbs: sustained clinical efficacy and prevention of ADA

The Paradigm for Successful Tolerance Induction as Defined by the Immune Tolerance Network

EpiVax



Huffaker MF et al (2021) Front. Immunol. 12:744804

Why Multiple Components are Necessary for Tolerance Induction: Safety and Efficacy Perspectives

EpiVax

- The potential for single agent antigen specific therapy to worsen disease: exacerbation of Multiple Sclerosis from treatment with an Altered Peptide Ligand (APL) immunized rather than tolerized (Bielekova B et al Nat Med 2000:1167-75)
- Documented failures to induce tolerance when sole focus is on Teff modulation: prolonged disease-free state but of limited duration.
- Limited patient populations for studies: consider the protocol most apt to induce clinically important outcomes; ***ensure study of potential mechanisms of tolerance induction in all studies***

Agenda

- Strategic staged approach for immune tolerance induction: focus on autoimmune disease
- From autoantibodies to autoimmune disease: the role of epitope spread, potential means to stop it and applicability to immunogenicity of biological therapeutics
- Clinical studies for preventing Type 1 Diabetes Mellitus in those at high risk: potential approaches for boosting and activating Tregulatory cells
- Novel modalities for treatment of autoimmunity: application to immunogenicity of biological therapeutics.
  - CD19 CAR-T cells
  - Therapeutic Drug Monitoring (TDM) of TNF mAbs: sustained clinical efficacy and prevention of ADA

MANDATE: STOP THE SPREAD!

EpiVax

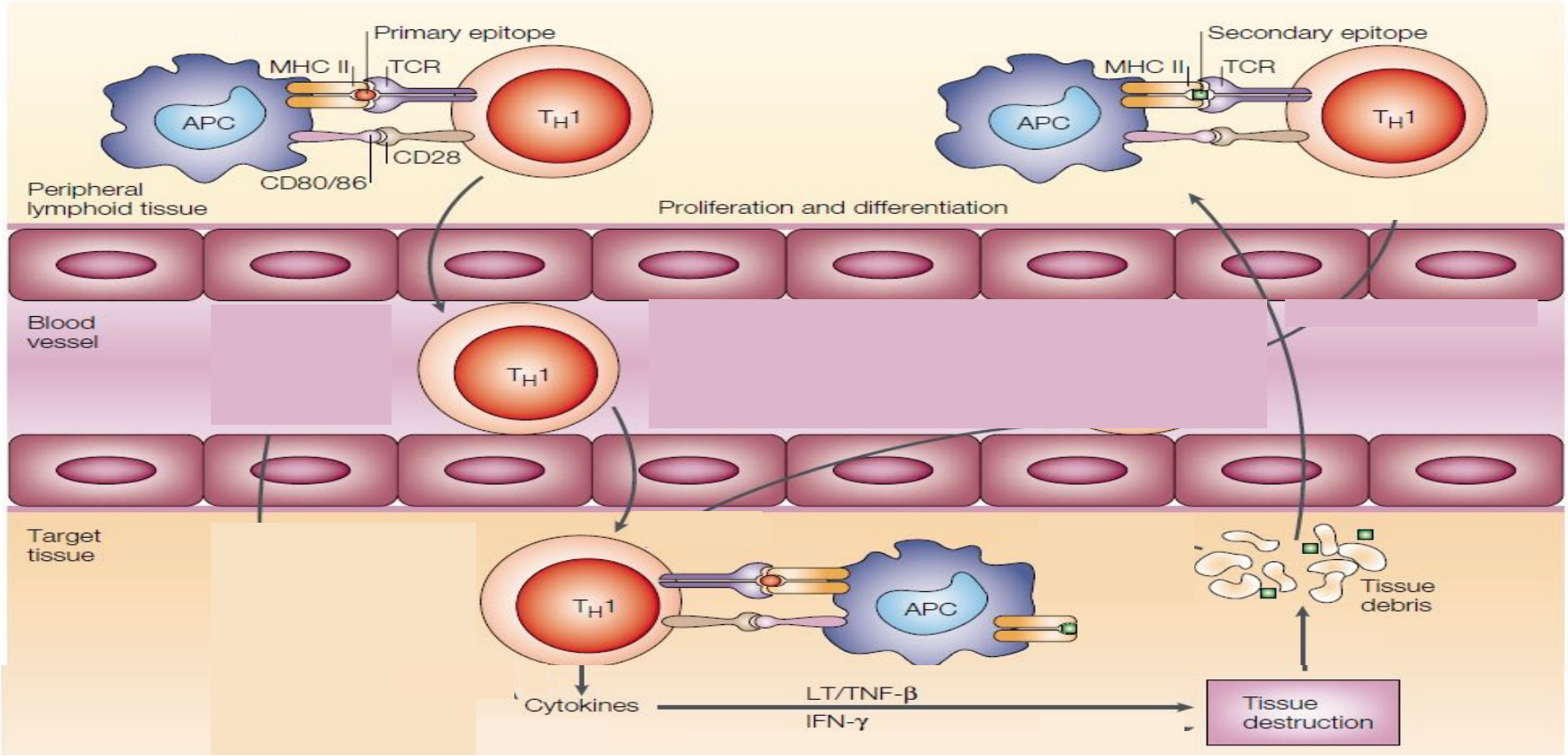
1) Autoimmune diseases such as T1DM and MS develop after a prolonged pre-clinical phase in which autoantibodies can be detected in patients at risk.

2) Epitope spread is key to induction of disease: intervention at the precursor stage could prevent progression to clinical disease and with less overall immune suppression.

3) Early detection is key to precluding disease
   1) In T1D, familial association identifies those at risk
   2) In MS, HLA DR15 haplotype is highest associated risk factor but HLA haplotypes not typically evaluated

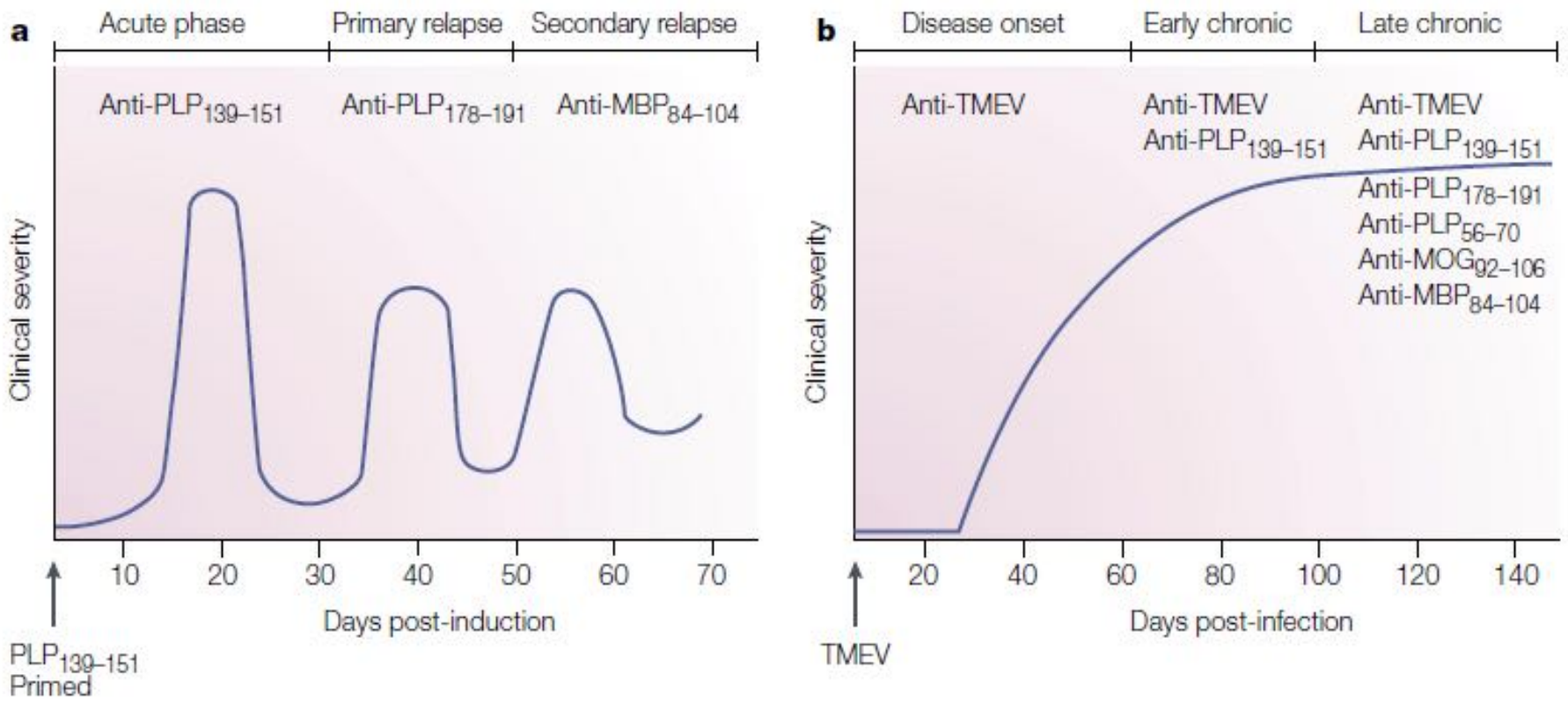
4) ***How to enhance early detection?***
   What public health measures may be beneficial and economically feasible?

Epitope Spread Facilitated by Tissue Destruction and Inflammation in Autoimmune and Virus-Induced Immunopathology



Adapted from Vanderlugt CL and Miller SD Nat Rev Imm 2002

Epitope Spread in T cells Mediating EAE and Viral Mouse Models of MS: From Intramolecular (PLP) to Intermolecular (PLP->MBP)



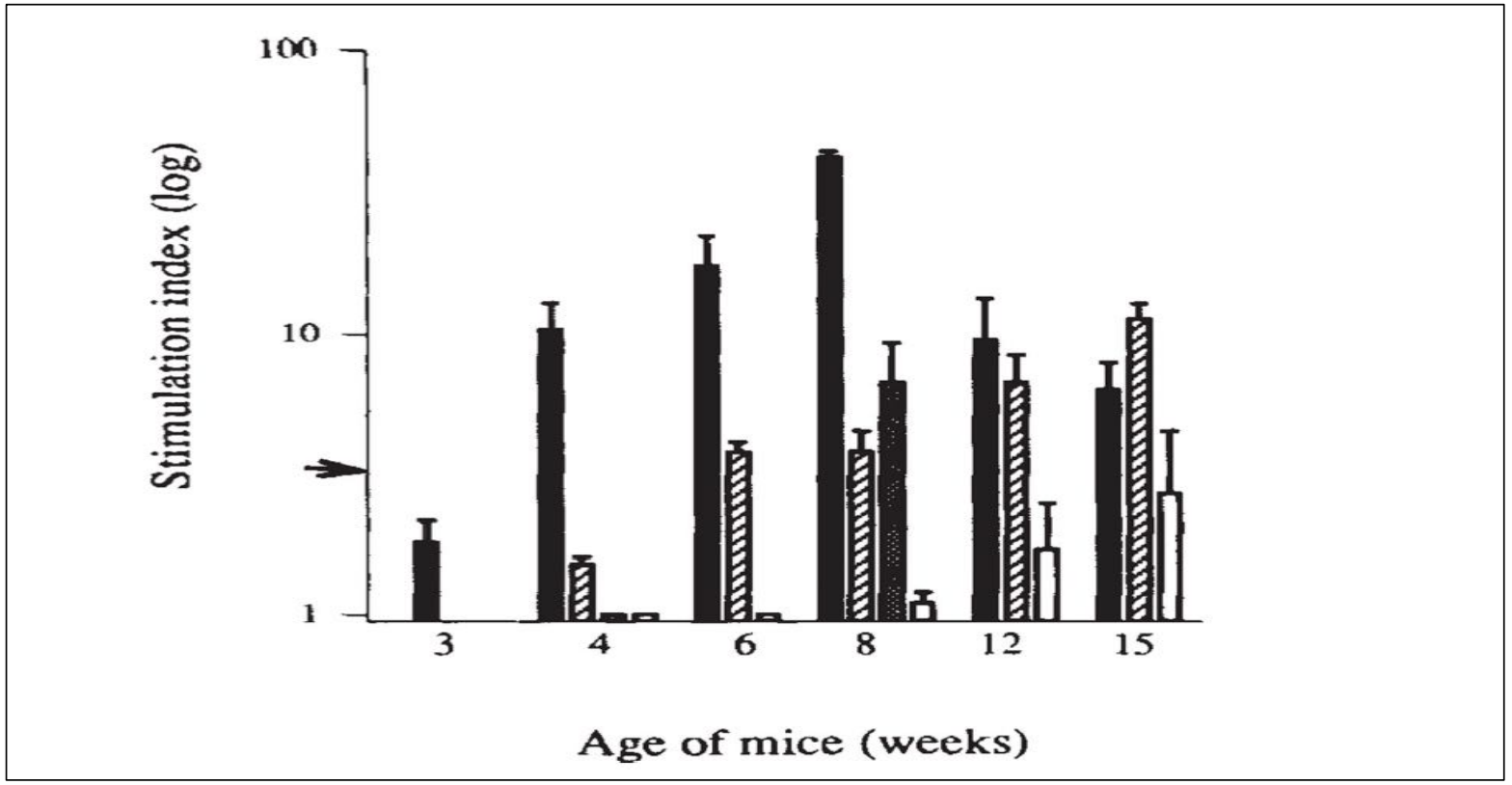
Vanderlugt CL and Miller SD Nat Rev Immunol 2002

T cell Epitopes in Human Neural Tissues Implicated in Multiple Sclerosis: High Avidity and Immunodominant

Green: + Association;
Red: - Association

Lutterotti A et al (2021) Front. Immunol. 12:640935.

T-cell Activation to Islet β-cell Antigens Develop Spontaneously and Evolve in a Defined Chronological Order in NOD mice



GAD-65

Hsp65

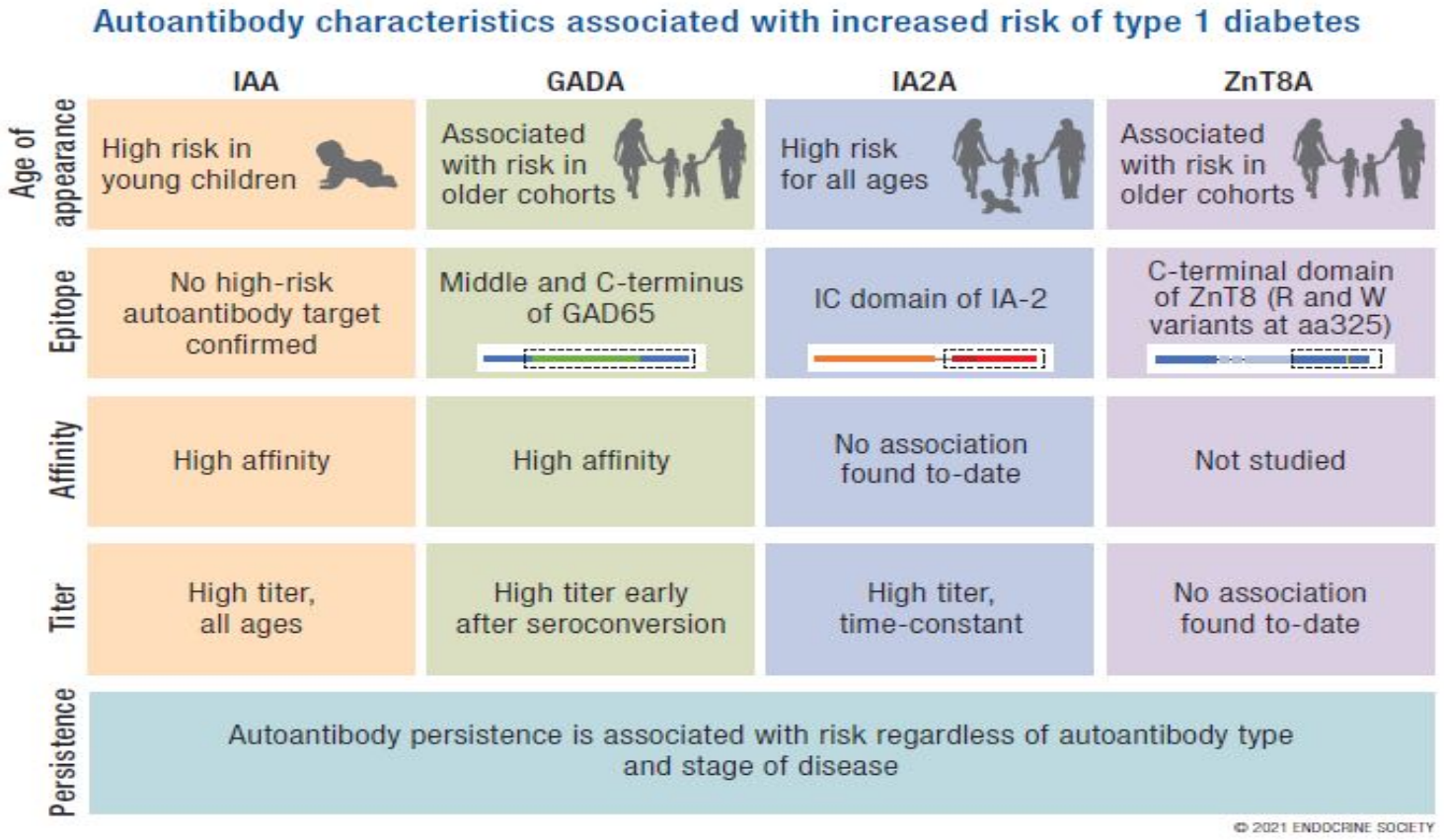
Carbox
-PEP H

insulin

Kaufman DL et al. Nature. 1993 November 04;
366(6450): 69–72. doi:10.1038/366069a0

Distinct Autoantibodies Identify Patients at High Risk of Type 1 DM: Window of Intervention to Prevent Progression to Disease?

EpiVax



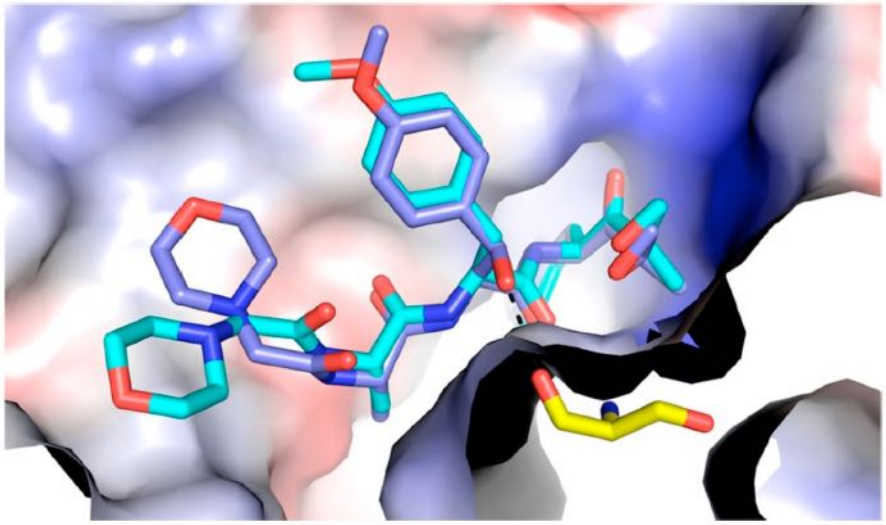
So, M et al Endocrine Reviews, 2021, Vol. 42, No. 5, 584–604

Stop Epitope Spread in Precursor Phases of Autoimmune Disease and in Early Treatment with Life-Saving Protein Therapeutics: the pillars

- ***Reduction of inflammation and further tissue damage***:
  - mAbs to inflammatory cytokines eg α-TNF, α-IL-12/23, α-IL-17
  - Diminish tissue damage (DAMP) induced innate immune responses.
  - Consider small molecule inhibitors of inflammation: eg JAK Inhibitors
- ***Immunoproteasome inhibition***
  - prevent antigen processing and presentation of tissue epitopes and biological therapeutics: targeting the subunits of the immunoproteasome; or global proteasome inhibition
- ***B cell depletion:***
  - interrupts antigen processing and presentation
  - prevents progression of precursor Ag specific B cells to antibody secreting plasma cells and B memory cells
- **Consider combination treatments for immune tolerizing strategies**

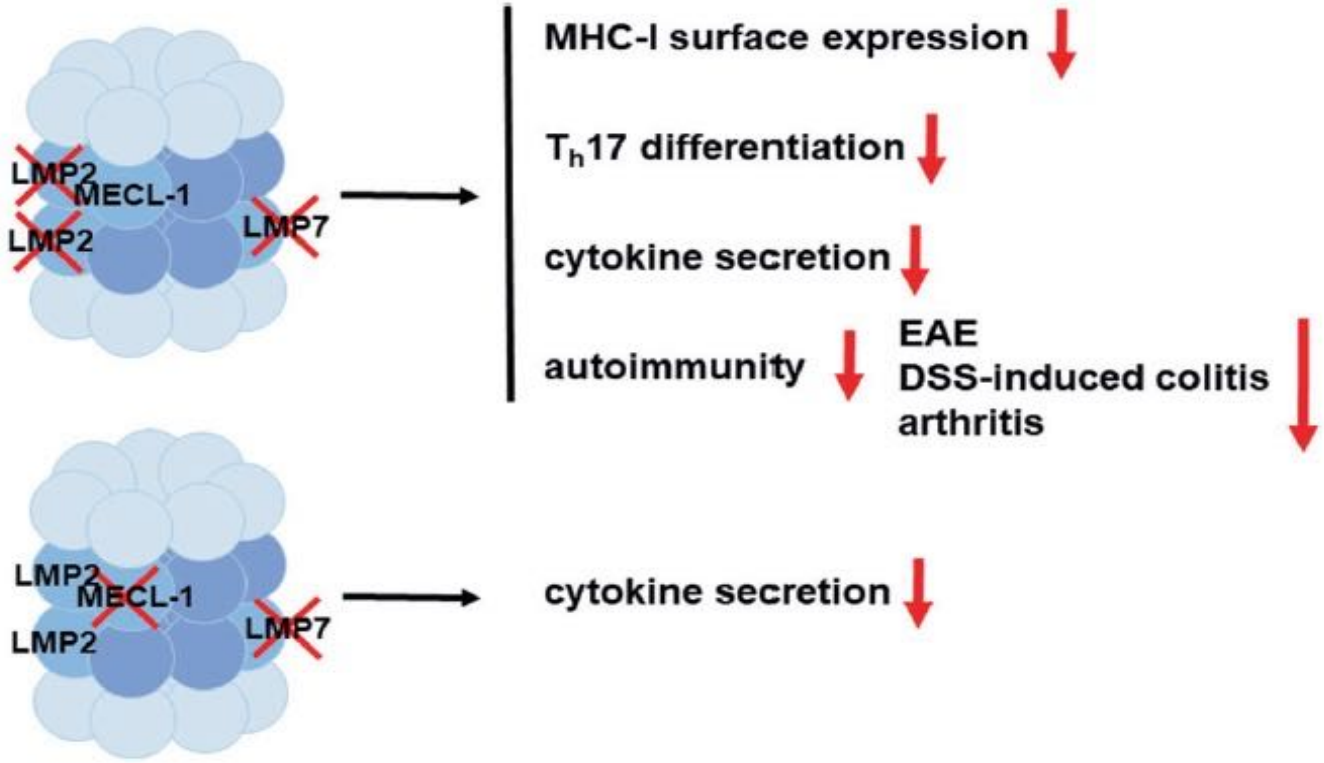
Design of Immunoproteasome Inhibitor Targeting LMP7 and LMP2 Subunits of the Immunoproteasome: KZR-616

EpiVax



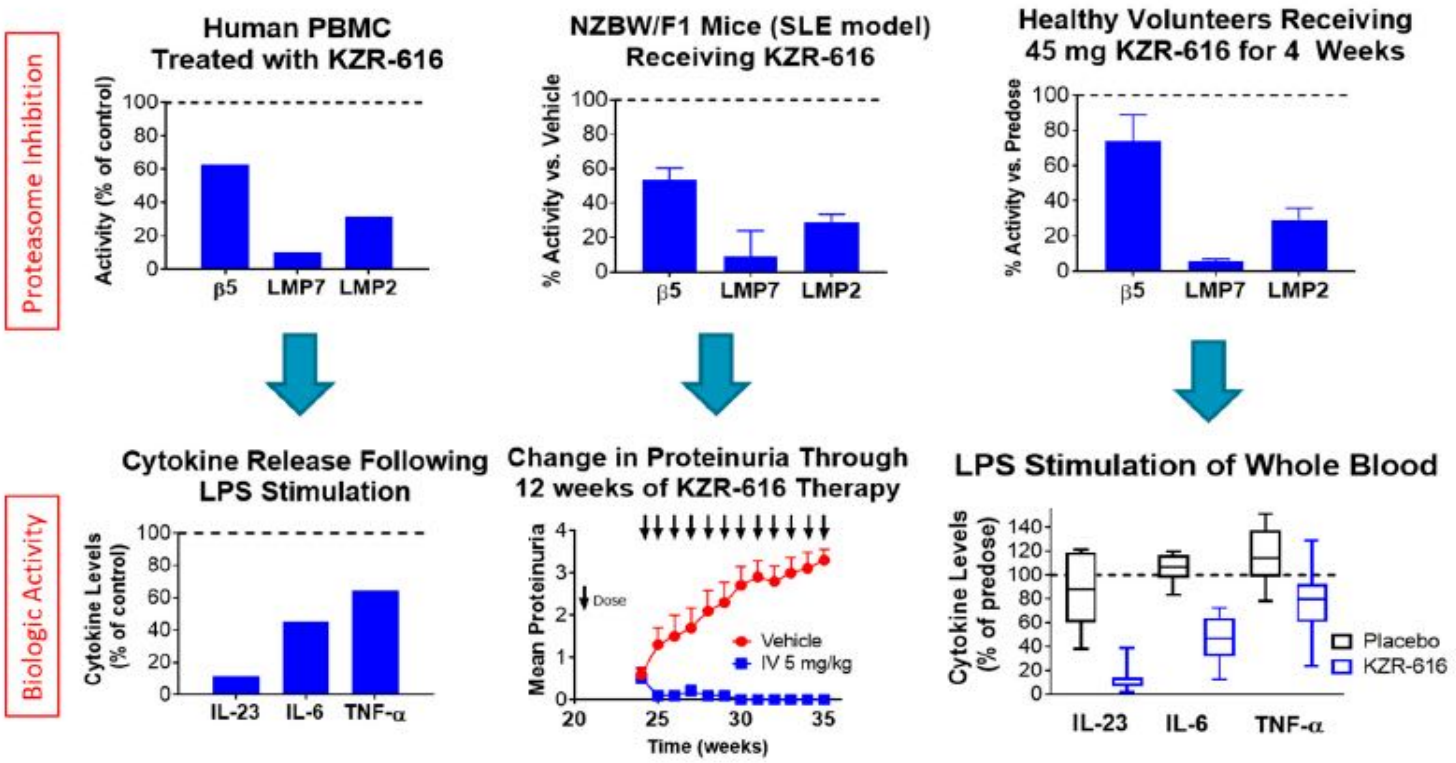
Kirk, C.J. et Cells **2022**, 11, 9.

Immunoproteasome Inhibition Ameliorates Autoimmunity in Mouse Models: the Mechanisms



M. Basler and M. Groettrup: Genes and Immunity 2020

Immunoproteasome Inhibition Phase 1 Study Marked Reduction in inflammatory Cytokines in PBL of Treated Healthy Volunteers



Kirk, C.J. et Cells **2022**, 11, 9.

Clinical Studies of Immunoproteasome Subunit Inhibitors for Treatment of Autoimmune Disease

EpiVax

- A Phase 2 randomized, double-blind, placebo-controlled, crossover multicenter study to evaluate the safety and efficacy of KZR-616 in the treatment of patients with active Polymyositis or Dermatomyositis (NCT04033926)
- MISSION STUDY: weekly administration of KZR-616 in patients with active proliferative Lupus Nephritis. Also, an open-label, dose escalation Phase 1b portion in SLE patients with or without nephritis (NCT03393013),
- A Study of KZR-616 in patients with Autoimmune Hepatitis (NCT05569759)
- A Study of KZR-616 in patients with active Lupus Nephritis (PALIZADE) (NCT05781750)

Immunoproteasome Inhibition to Prevent ADA to Life Saving/Chronically Administered Therapeutic Biologics?

- When given together with a protein therapeutic, can it block generation of immunostimulatory T cell epitopes? ***Caveat***: may also reciprocally block generation of regulatory T cell epitopes.

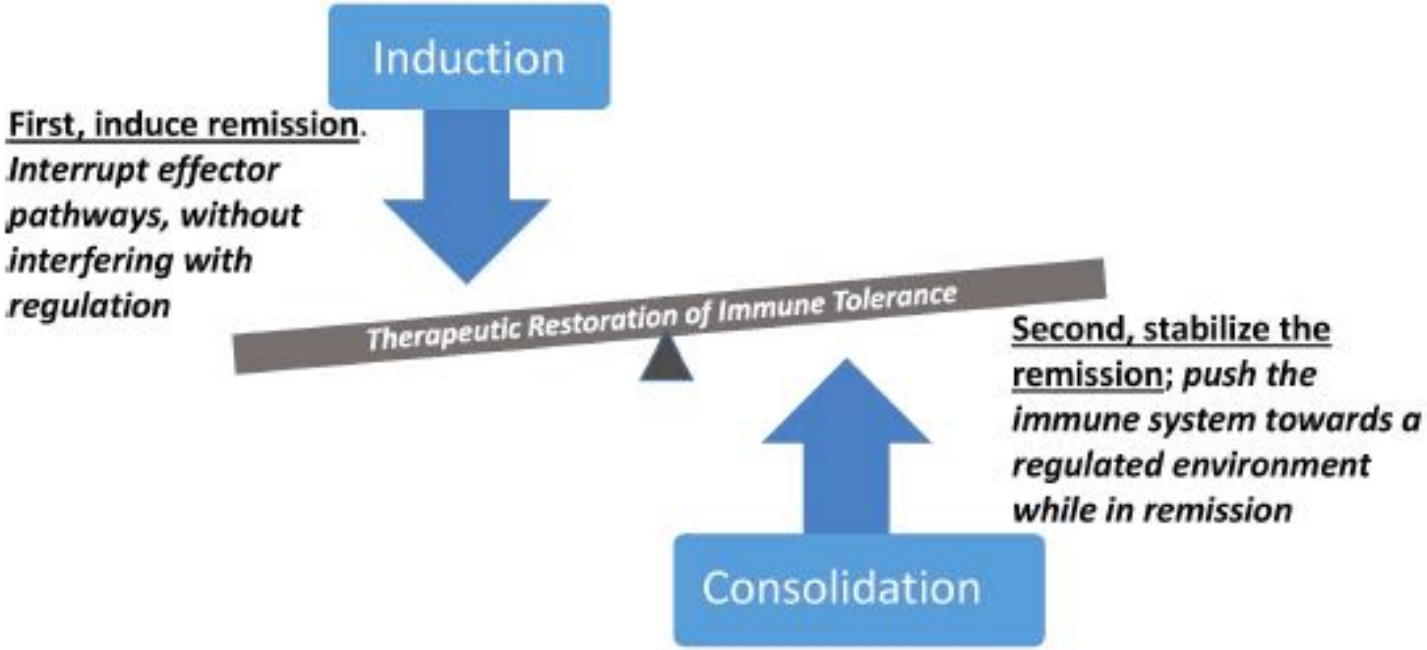
- CDRs of mAbs are the primary targets of ADA .Treatment with immunoproteasome inhibition of consideration early in treatment to prevent lack or loss of efficacy for mAbs.

- Is there a window for halting epitope spread from initial detection of ADA to blocking increase in titer and development neutralizing antibodies?
  - ADA->-> isotype switching+affinity maturation->> epitope spread →NABs?

Agenda

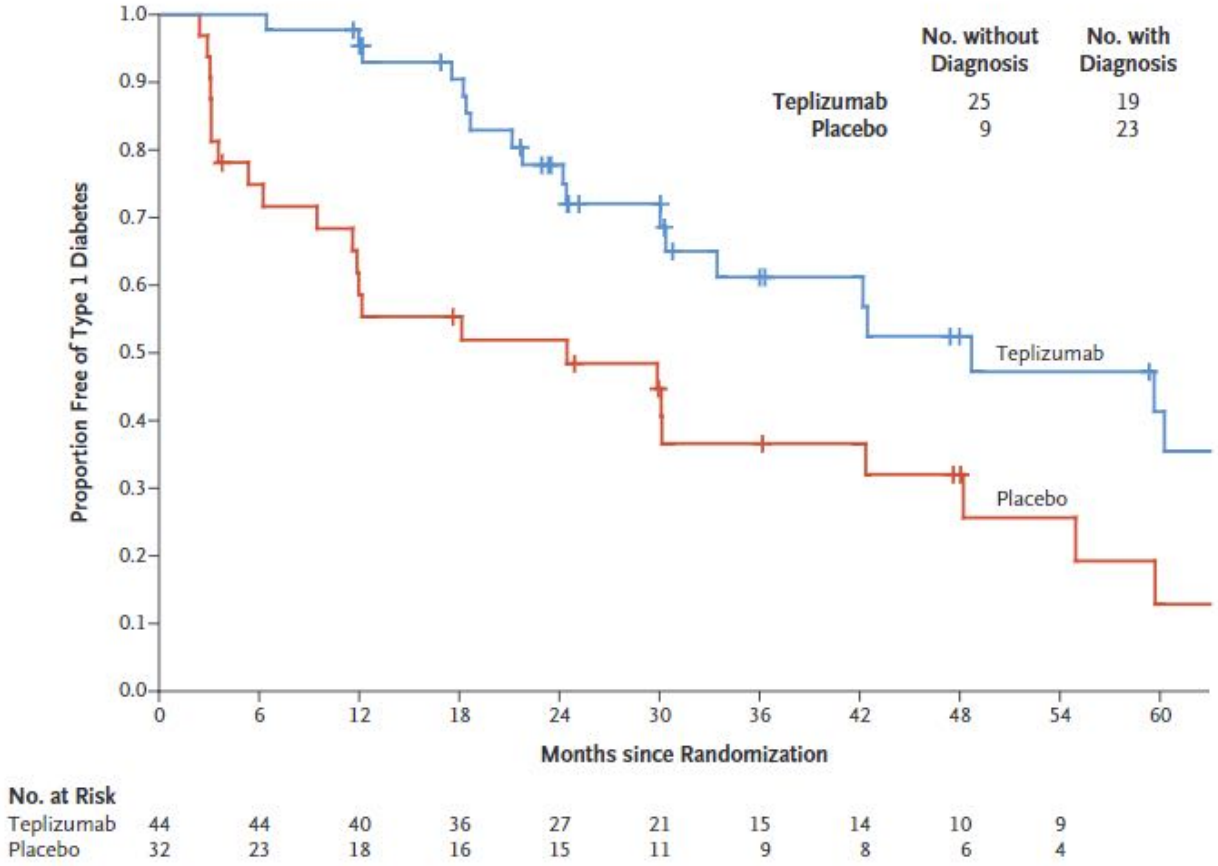
- Strategic staged approach for immune tolerance induction: focus on autoimmune disease
- From autoantibodies to autoimmune disease: the role of epitope spread, potential means to stop it and applicability to immunogenicity of biological therapeutics
- Clinical studies for preventing Type 1 Diabetes Mellitus in those at high risk: potential approaches for boosting and activating Tregulatory cells
- Novel modalities for treatment of autoimmunity: application to immunogenicity of biological therapeutics.
  - CD19 CAR-T cells
  - Therapeutic Drug Monitoring (TDM) of TNF mAbs: sustained clinical efficacy and prevention of ADA

Clinical study of Teplizumab a non-FcR binding CD3 mAb to interrupt T1D effector cells



Nepom GT (2022) .Front. Immunol. 13:962177.

Teplizumab Significantly Prolongs Interval to Onset but does not Prevent Type 1 Diabetes in Patients at High Risk



Median time to diagnosis of type 1 diabetes:

48.4 months in the teplizumab group

24.4 months in the placebo group

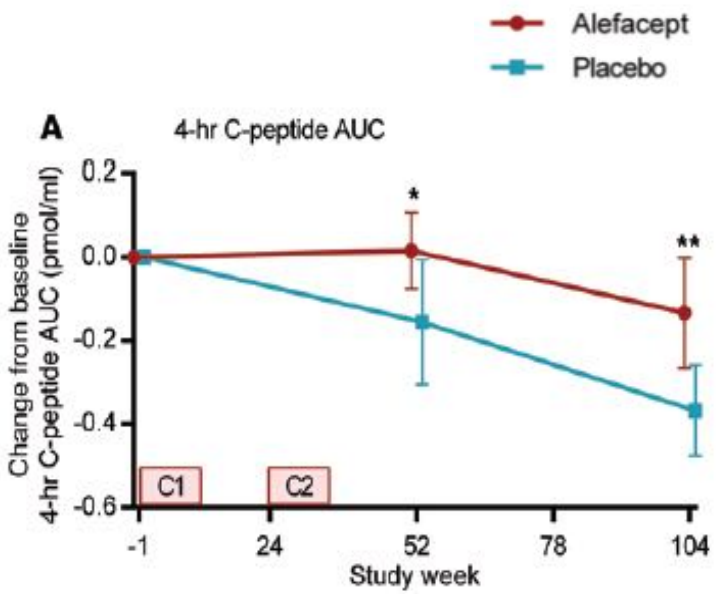
FDA Approved 2022

Herold et al. N Engl J Med. 2019 August 15

Alefacept (LFA-3-Ig) Binds to CD2 on T Cells: Significant but Limited Preservation of Beta Cell Function in Recent Onset T1D

T1DAL
ITN045AI | Inducing Remission in New Onset T1DM with Alefacept (Amevive®)

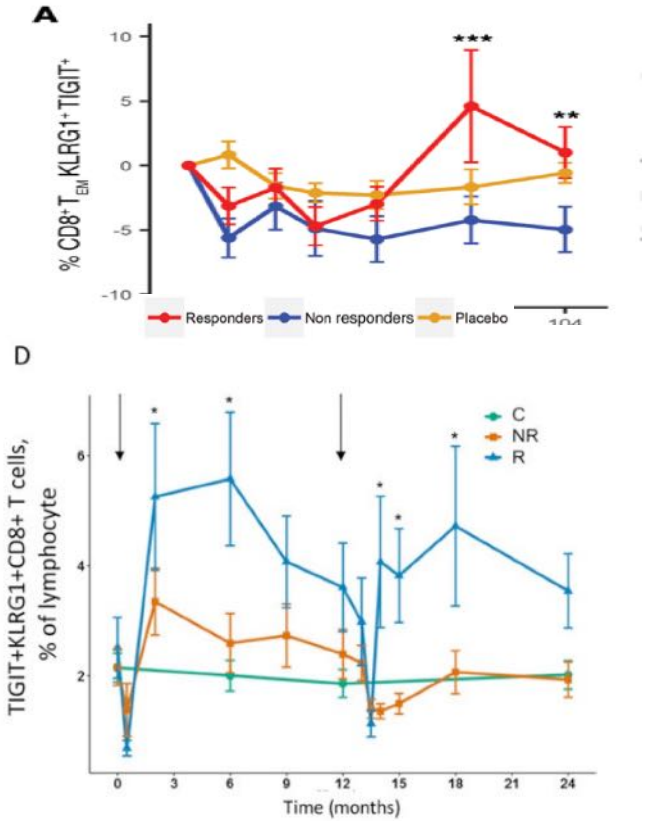
- Alefacept (LFA-3-Ig) is a fusion protein that binds to CD2: ***expressed at highest levels on T effector memory cells***.
- Treatment of ***newly diagnosed T1D*** patients with alefacept
  - sustained preservation of pancreatic beta cell function
  - reduced insulin usage
  - reduced hypoglycemic episodes.
- Effect sustained for months after alefacept treatment but waned over time.



Rigby et al (2015) JCI 125: 3285

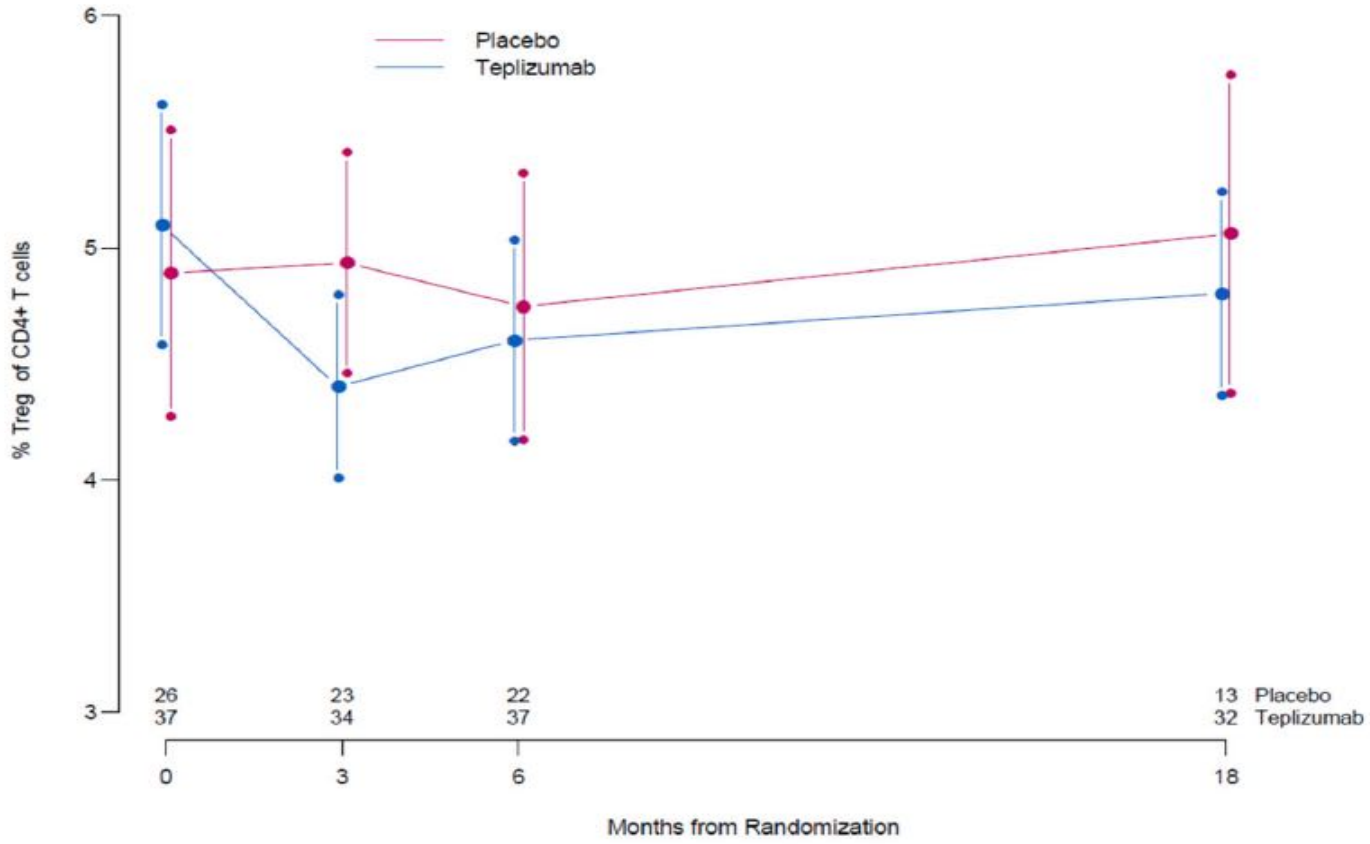
Responders to Teplizumab and Alefacept Show "Exhausted" Signature of CD8+T effector Memory Cells

KLRG1+ TIGIT+ CD8+ Tem "exhaustion" signature associated with pancreatic beta cell preservation in Alefacept (top) and Teplizumab (bottom) Responders



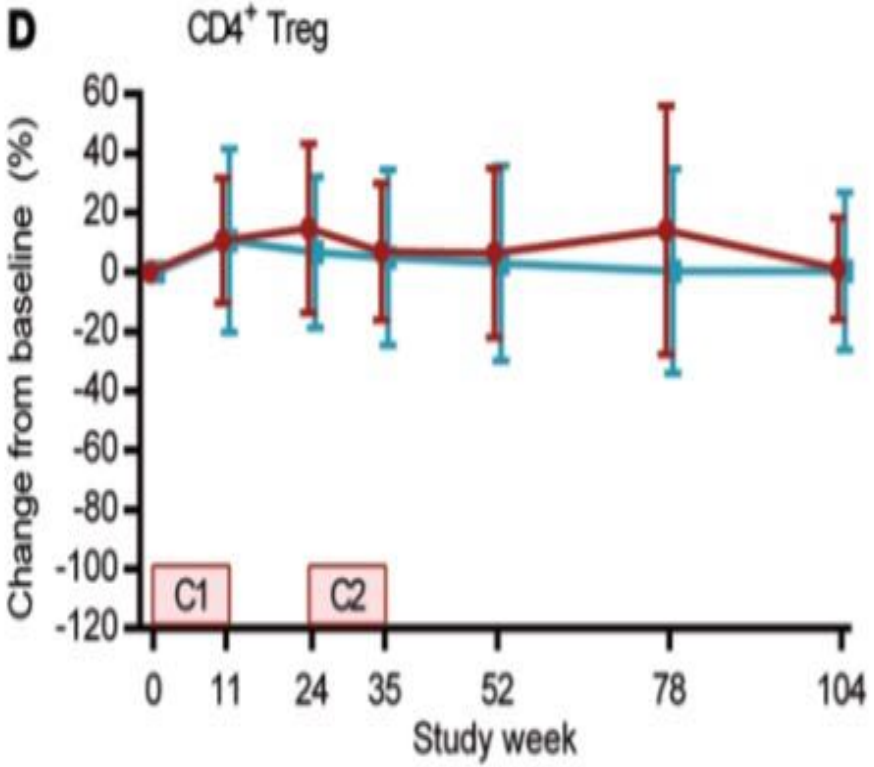
Diggins et al 2021 JCI Insight https://doi.org/10.1172/jci.insight.142680
Long SA et al 2016 Sci Immunol. doi:10.1126/sciimmunol.aai7793

The Culprit: Failure to Increase the Frequency of *CD4+ Tregs* Over Placebo Treated Patients



Herold et al. N Engl J Med. 2019 August 15

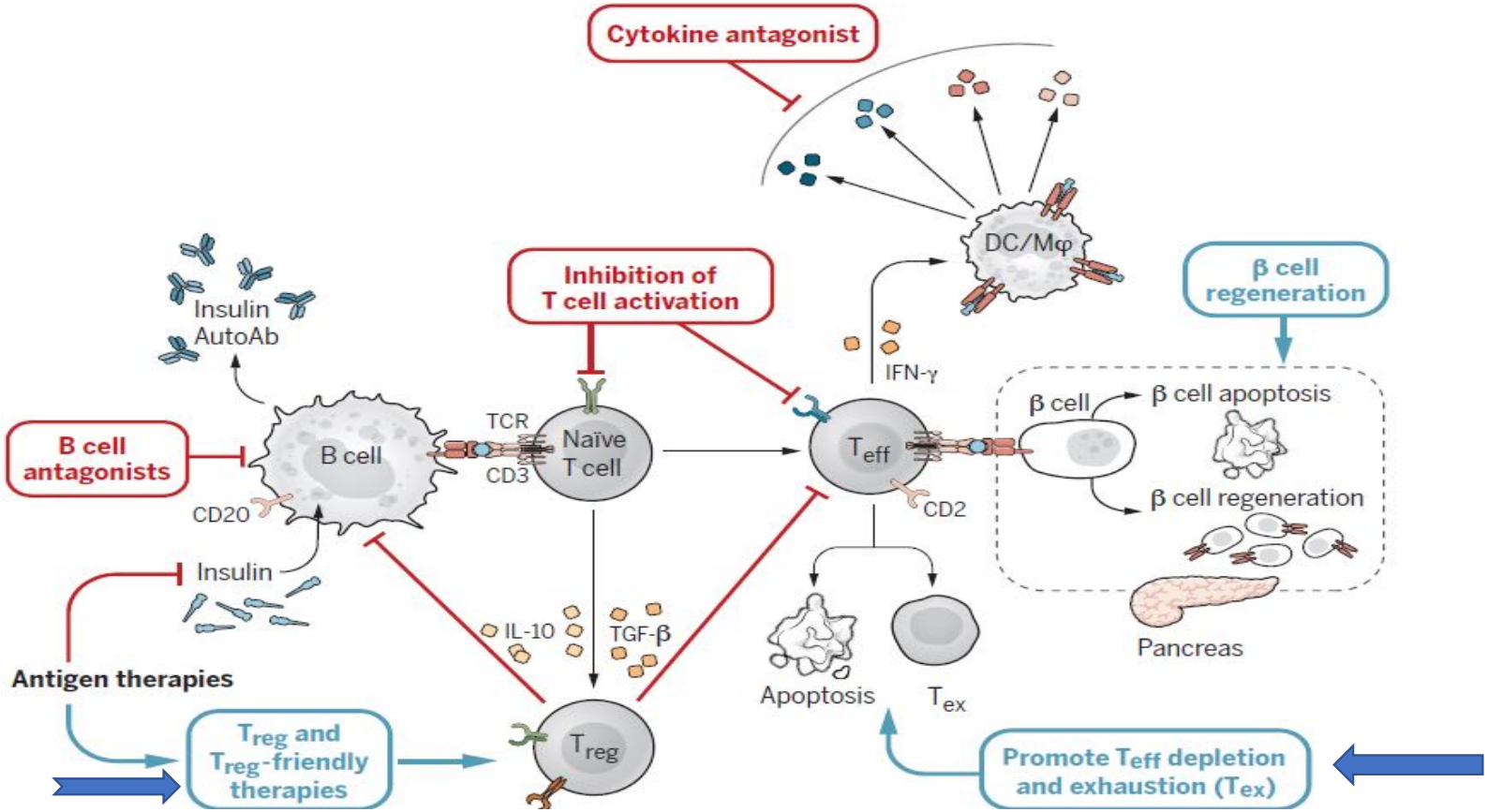
Immune Modulation with Alefacept Neither Induces nor Boosts Tregs



T regulatory cells are preserved but not enhanced.

Rigby et al (2015) JCI 125: 3285

Optimal Outcome: Combine T cell Exhaustion with Induction and Activation of Tregs and β cell Regeneration for T1D



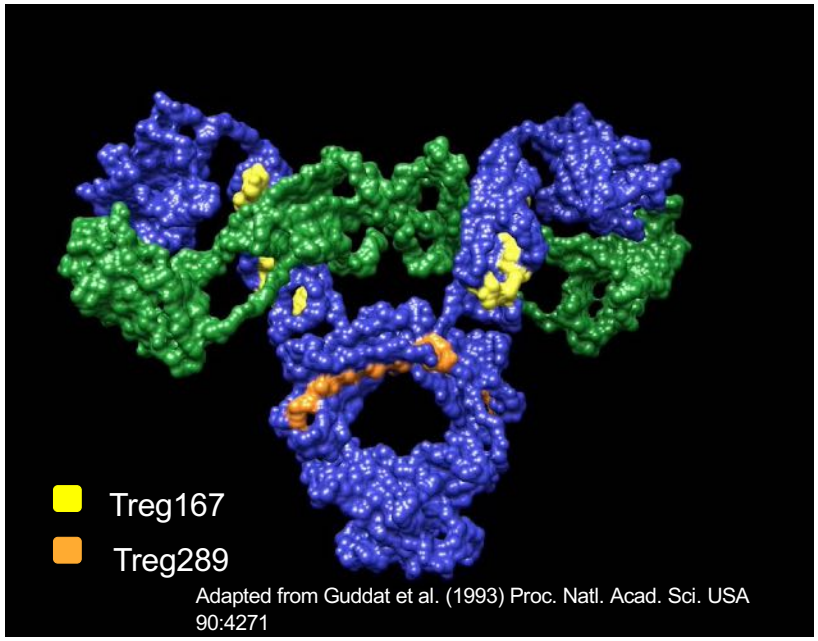
Bluestone et al., Science 373, 510–516 (2021)

“Treg Friendly Therapies”

EpiVax

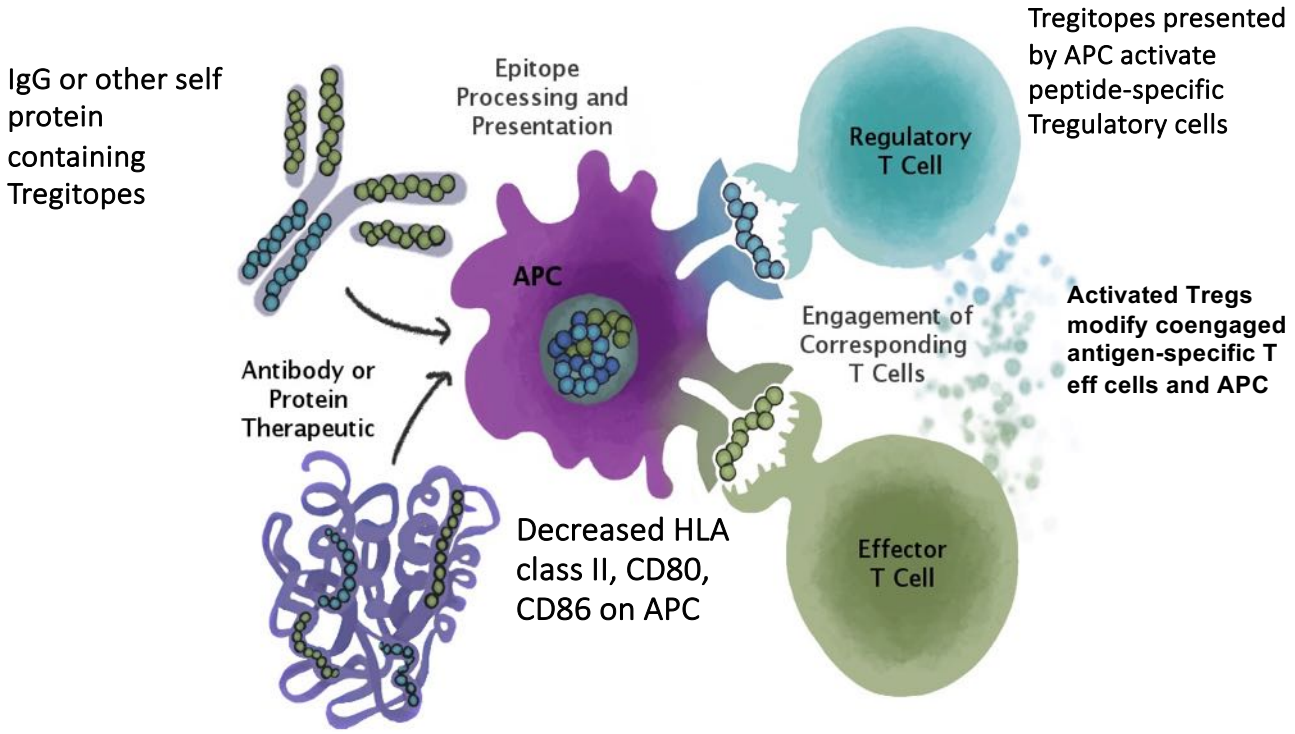
- Tregulatory Cell Populations:
  - TcR transgenic Tregs
  - CAR and BAR Treg cell populations
- Therapeutics to generate tolerogenic APC and induction of Tregs
  - Regulatory T cell epitopes: Tregitopes
  - Rapamycin/Rapamycin Nanoparticles
  - Cytokines/anti-cytokine mAbs: single or combinations eg IL-2 engineered to bind only IL-2Rα, IL-10, TGF-β. Couple with anti-cytokine mAbs to inflammatory cytokines
- Liver Depot Gene Therapy

Regulatory T cells Epitopes (Tregitopes) Present in Immunoglobulin IgG Conserved Domains



- 15-20 aminoacid peptides in conserved IgG regions
- Tregitopes are presented by multiple HLA Class II molecules
- Highly conserved among IgG molecules across species: potential basis for IVIG induced regulation
- Induce natural Tregs to modify immune responses

Tregitopes Activate Tregs: Modify Effector Function of Teff cells and Generate Tolerogenic APC



- **Tregitopes**: Short, linear peptide sequences that bind to HLA and activate ***regulatory T cells***
- Identified by Epivax immunoinformatics searching on epitopes that are homologous across human proteome at the TCR face
- ***Can be co-formulated or attached to immunogenic proteins to provide antigen-specific tolerance***
- Wide range of therapeutic applications
- Two mechanisms of action:
  - Effects directly on Tregs
  - Effects on APC

De Groot AS et al Expert Rev. Clin. Pharmacol. 6(6), 651–662 (2013)

Agenda

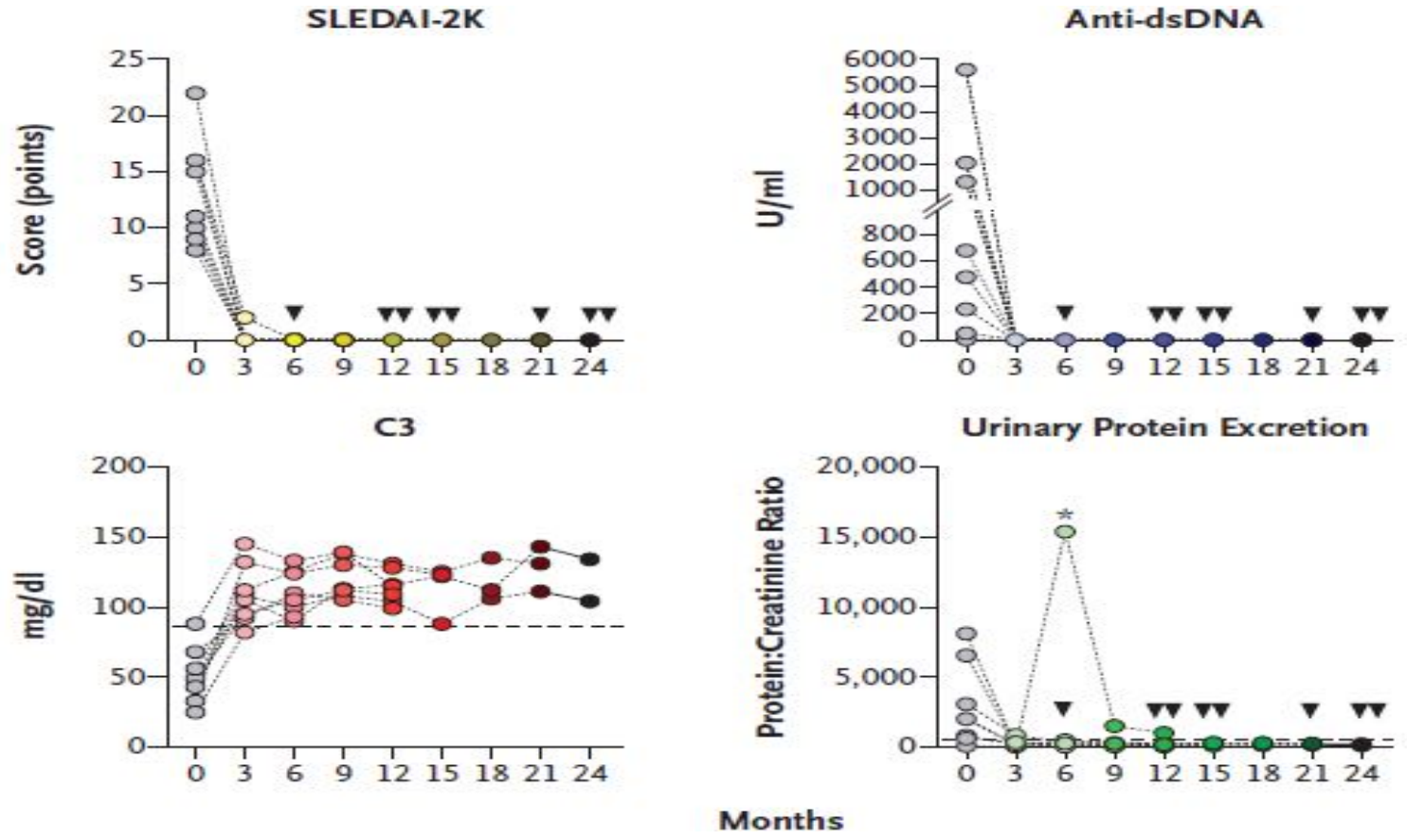
- Strategic staged approach for immune tolerance induction: focus on autoimmune disease
- From autoantibodies to autoimmune disease: the role of epitope spread, potential means to stop it and applicability to immunogenicity of biological therapeutics
- Clinical studies for preventing Type 1 Diabetes Mellitus in those at high risk: potential approaches for boosting and activating Tregulatory cells
- Novel modalities for treatment of autoimmunity: application to immunogenicity of biological therapeutics.
  - CD19 CAR-T cells
  - Therapeutic Drug Monitoring (TDM) of TNF mAbs: sustained clinical efficacy and prevention of ADA

Breakthrough Therapeutic: CD19 CAR-T Cells for Treatment of Autoimmune Diseases-A Magic Bullet?

- Rituximab has poor efficacy in SLE despite recurrent dosing
  - Upregulation of BAFF following depletion by rituximab associated with increased flares, increased dsDNA antibodies and faster repopulation of B cell populations
  - Alleviated to an extent by treatment with belimumab
- Study: CD19 CAR-T cells for Treatment of Patients with severe SLE, Scleroderma, Inflammatory Myositis
  - Single infusion of CD19 CAR T cells: 8 patients with severe SLE, 3 patients with idiopathic inflammatory myositis and 4 patients with systemic sclerosis who received a single infusion
- Patients all preconditioned with fludarabine and cyclophosphamide
- Safety: No moderate- or high-grade cytokine release syndrome (some patients treated with Tocilizumab) or Immune effector cell-associated neurotoxicity syndrome; 1 serious infection but infections were mostly mild upper respiratory tract infections
- Efficacy assessed up to 2 years after CAR T-cell infusion by Clinical Disease Specific Scales: full B-cell reconstitution for up to 2 years without disease relapse

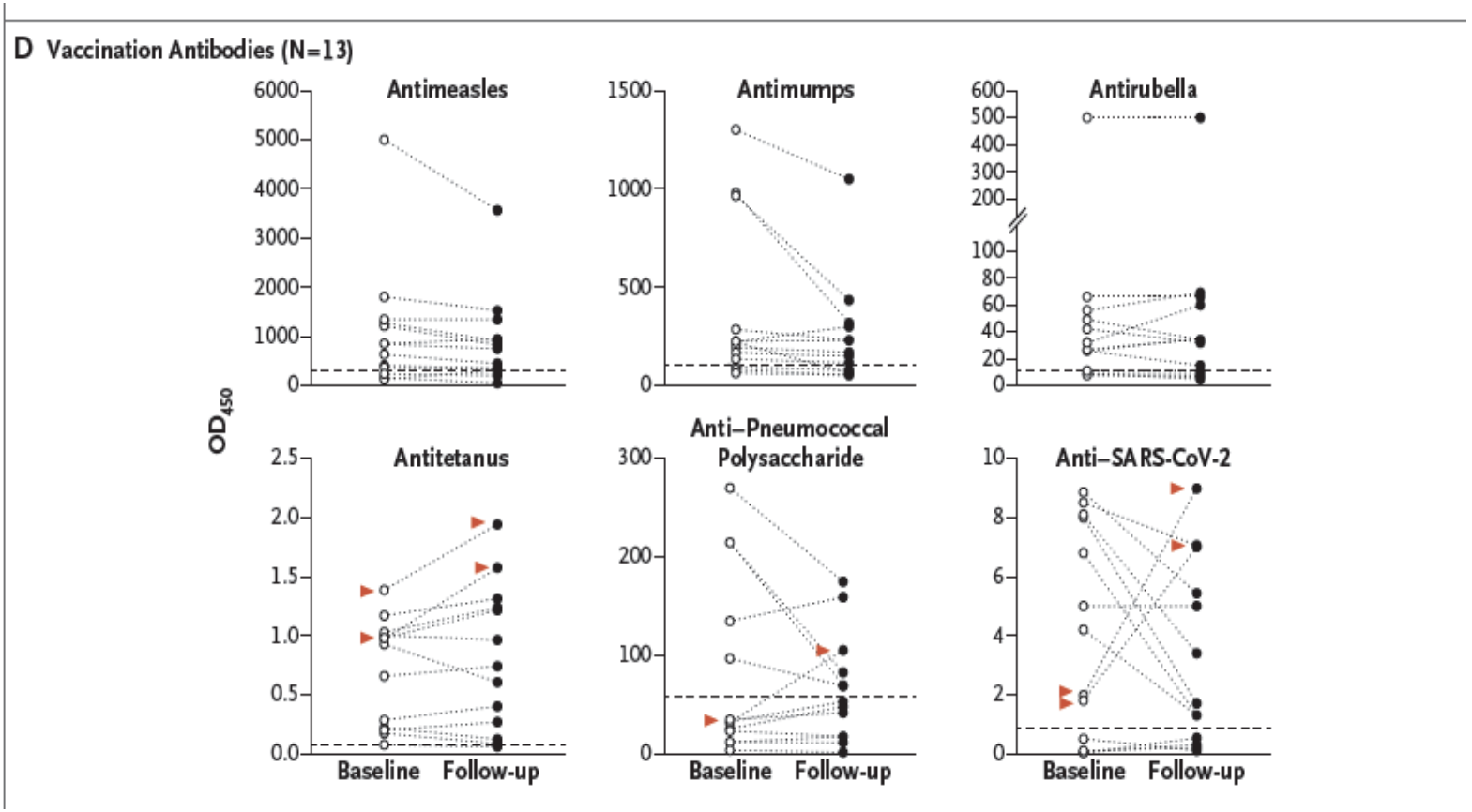
Muller F et al N Engl J Med 2024;390:687-700.

Are CD19 CAR-T cells a "Magic Bullet" for Autoimmune Diseases Marked by Autoantibodies?



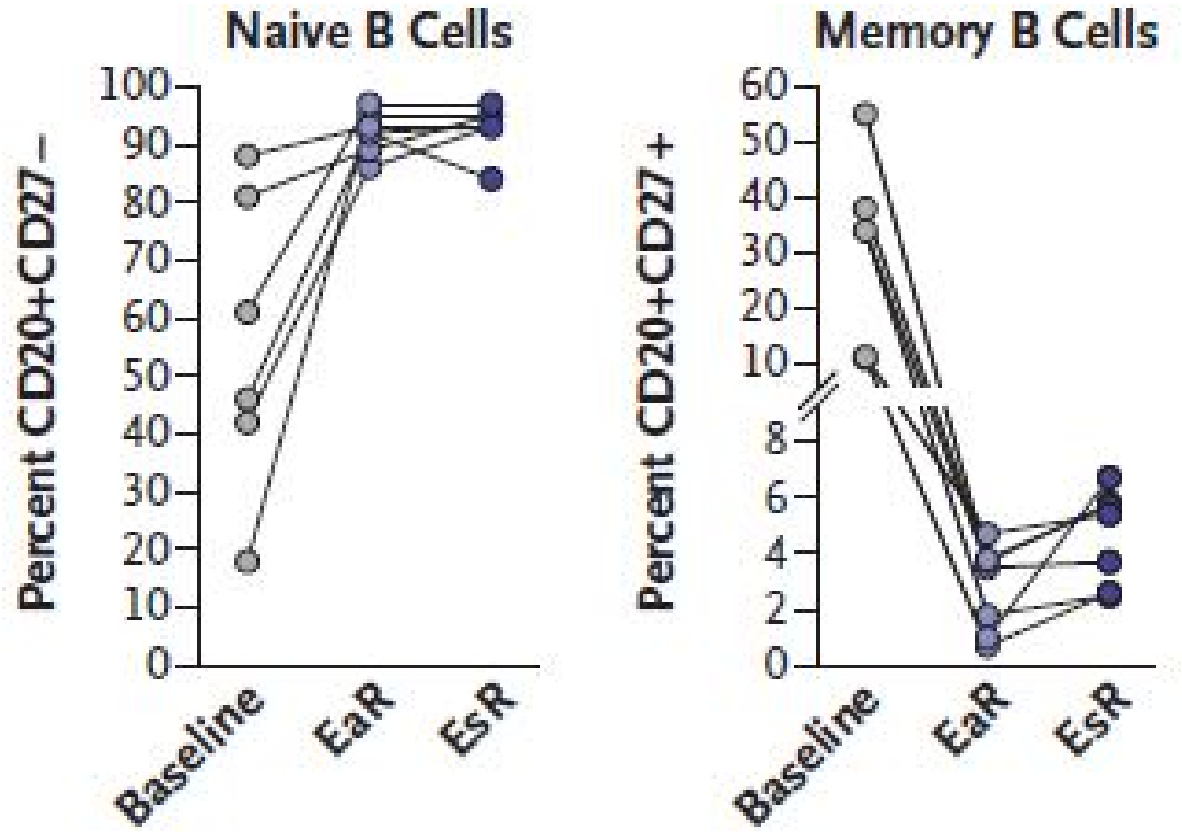
Muller F et al N Engl J Med 2024;390:687-700.

Safety: Vaccine Antibodies Generally Maintained at Critical Level in Patients treated with CD19 CAR-Ts



Muller F et al N Engl J Med 2024;390:687-700.

Prolonged Depletion of Memory B Cells but Rapid Return of Naïve B Cell Population Under Immunomodulatory Conditions

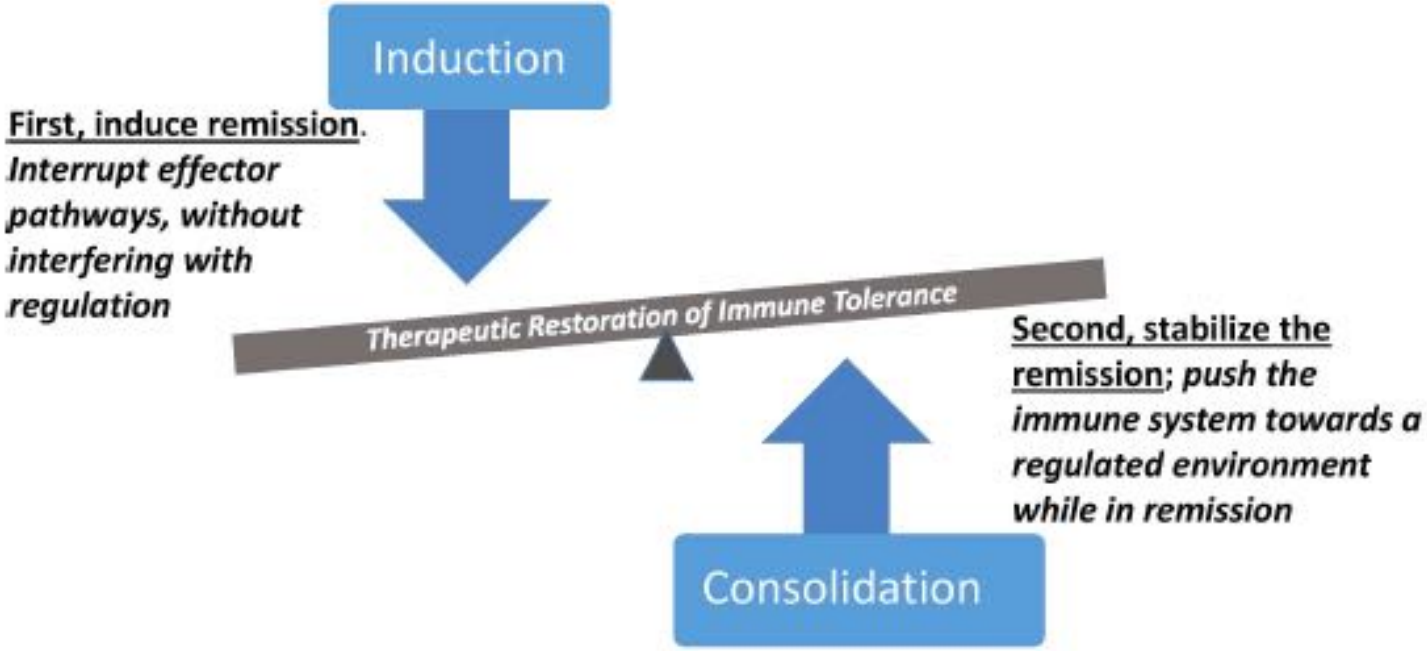


EaR: early reconstitution ~ 4 months post-treatment

EsR: reconstitution 1 year post- treatment.

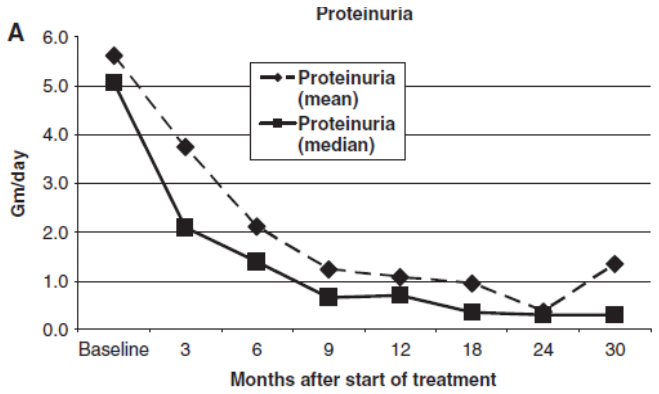
Muller F et al N Engl J Med 2024;390:687-700.

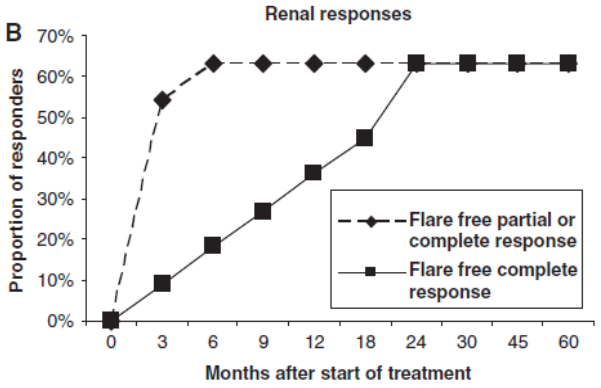
Patients Preconditioned with Fludarabine and Cytoxan: Did this "Set the Stage"?

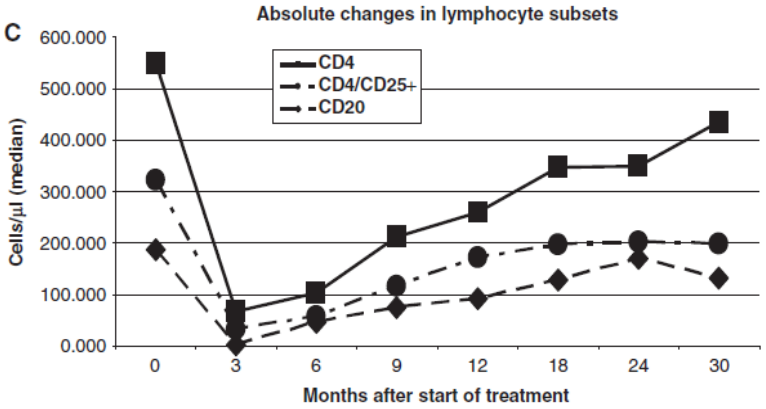


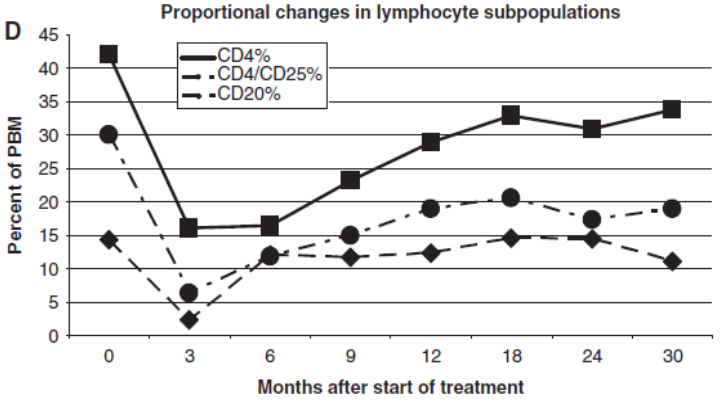
Nepom GT (2022) .Front. Immunol. 13:962177.

Fludarabine and Cytoxan Preconditioning had Profound Effects on T and B cell Populations and Disease Prior to CD19 CAR T Cells







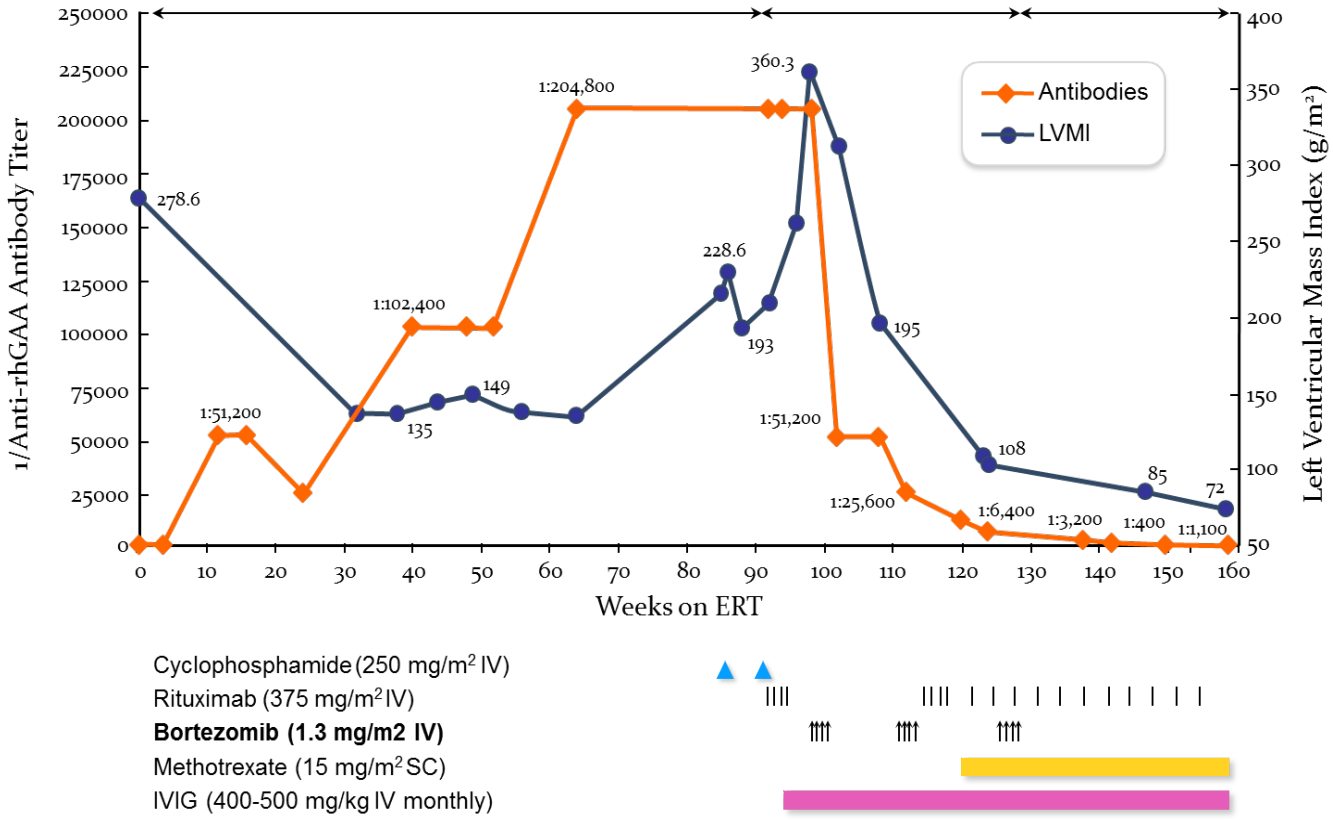


Illei, GG et al Rheumatology 2007;46:952–956

Key Question: is Immune Tolerance Induced?

- **Does a single course of preconditioning plus CD19 CAR-T induce tolerance?**
- **If so, what is the mechanism(s) of immune tolerance?**
  1) Active Immune Tolerance
     - Tregs
     - Bregs
  2) Anergy: immune checkpoint molecule expression on autoreactive T cells?
  3) Deletion
- **Essential to define mechanism.**
- **Is tolerance concomitantly induced to "innocent" or pathogenic bystanders?**
- **Durability: effects of intercurrent infection or trauma**

Could CD19-CAR T Cells Replace Multi Drug Prolonged Immune Suppressive/Tolerizing Regimen to Eliminate Life Threatening ADA?



Tolerance induction requires not only elimination of plasma cells but continued treatment with Rituximab, Methotrexate and IVIG

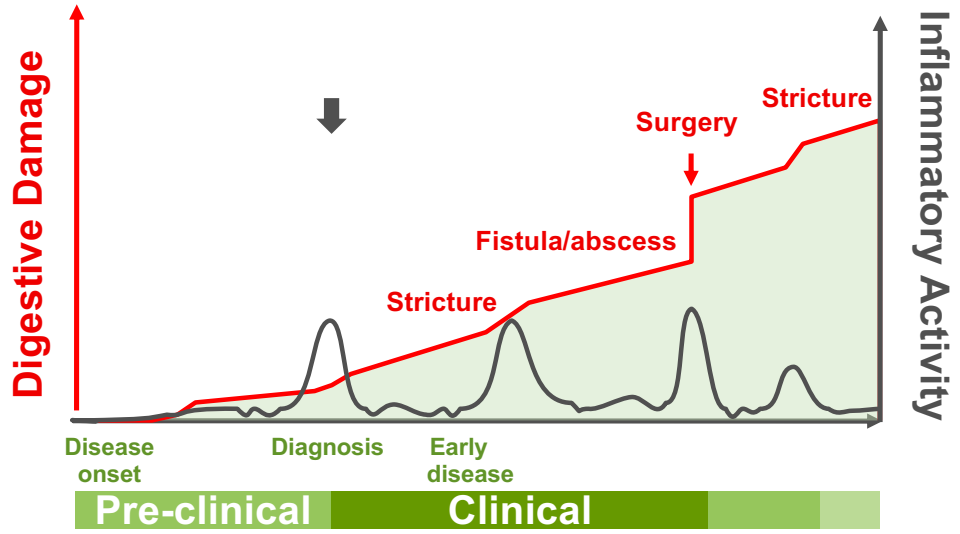
(Kishnani PS et al 2012)

Agenda

- Strategic staged approach for immune tolerance induction
- From autoantibodies to autoimmune disease: the role of epitope spread and potential means to stop it: applicability to immunogenicity of biological therapeutics
- Clinical studies for preventing Type 1 Diabetes Mellitus in those at high risk: necessity for boosting and activating Tregulatory cells
- Novel modalities for treatment of autoimmunity and prevention/elimination of ADA
  - CD19 CAR-T cells
  - Therapeutic Drug Monitoring (TDM) for TNF mAbs

Inflammatory Bowel Disease is Progressive, Destructive, and Carries a High Risk of Surgery and Colostomy

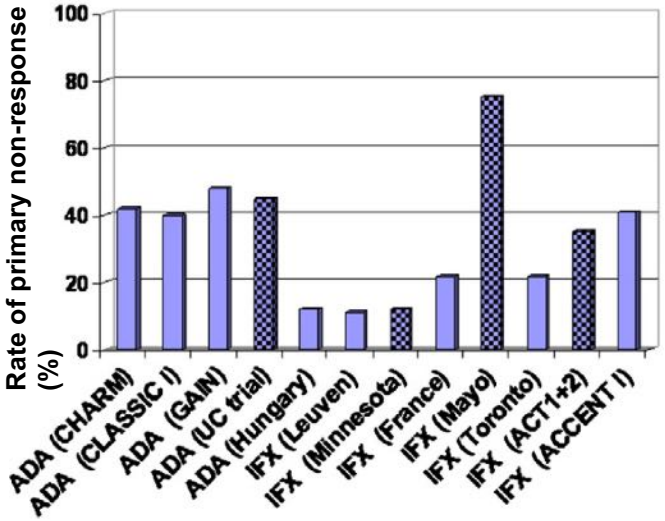
Progression of Digestive Damage and Inflammatory Activity in a Theoretical CD Patient



CD = Crohn's disease; CDAI = Crohn's disease activity index; CDEIS = Crohn's disease index of severity; CRP = C-reactive protein.

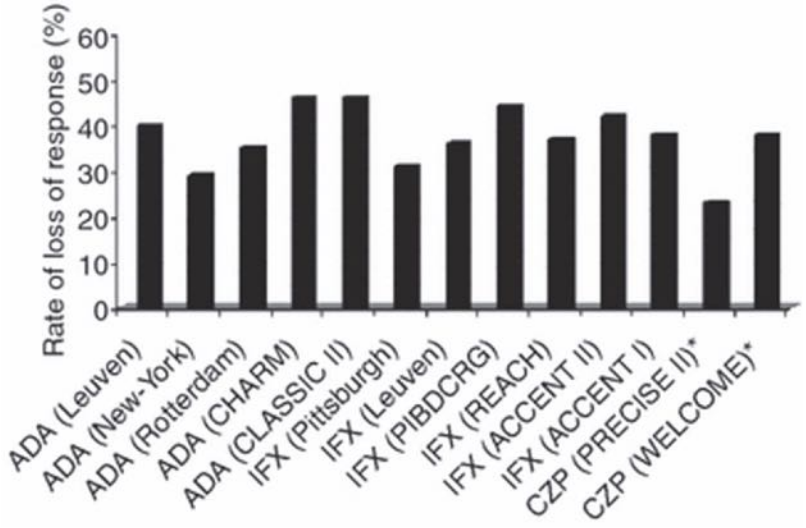
Pariente B, et al. *Inflamm Bowel Dis*. 2011;17(6):1415-1422

mAbs to TNF Have Revolutionized the Treatment of IBD but High Rate of Secondary Loss of Response due to ADA



IFX: infliximab

ADA: adalimumab



IFX: infliximab

ADA: adalimumab

CZP: certolizumab pegol

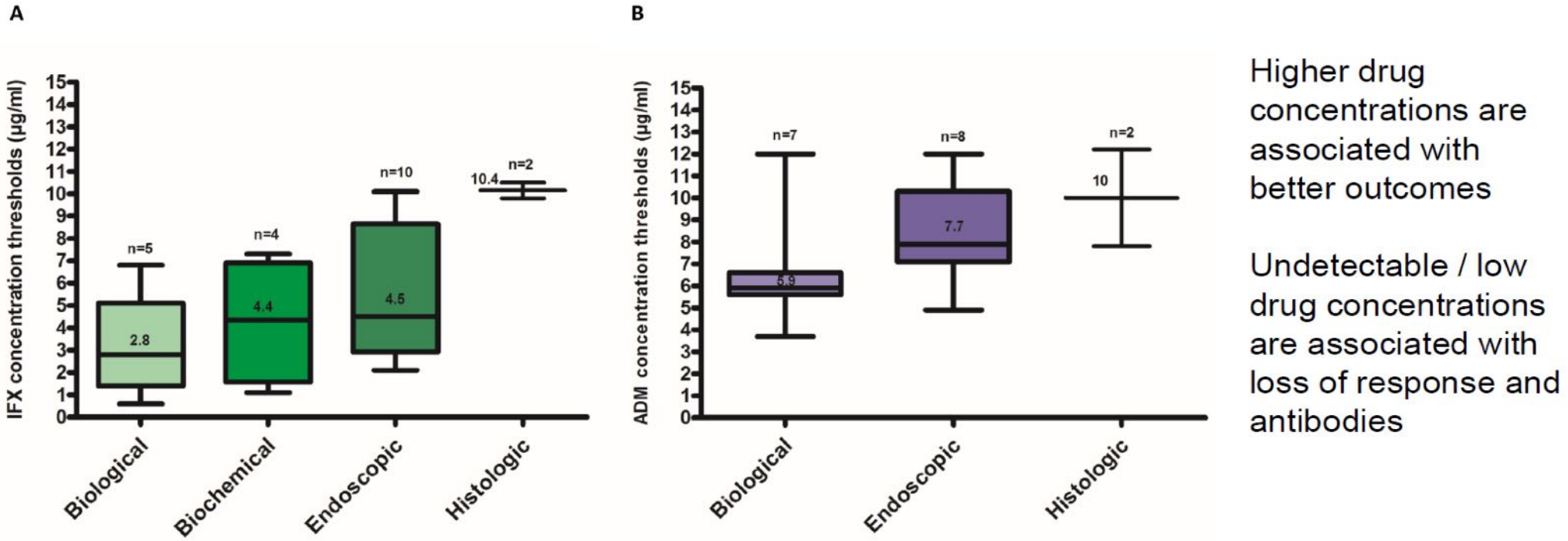
Ben-Horin and Chowers Aliment Pharmacol Ther 2011;33:987-95

Ben-Horin et al. Autoimmun Rev 2014;13:24-30

Maintenance of High Drug Concentrations of TNF mAbs Enhance Mucosal Healing in IBD

EpiVax

Infliximab and adalimumab concentration thresholds associated with objective therapeutic outcomes in inflammatory bowel disease



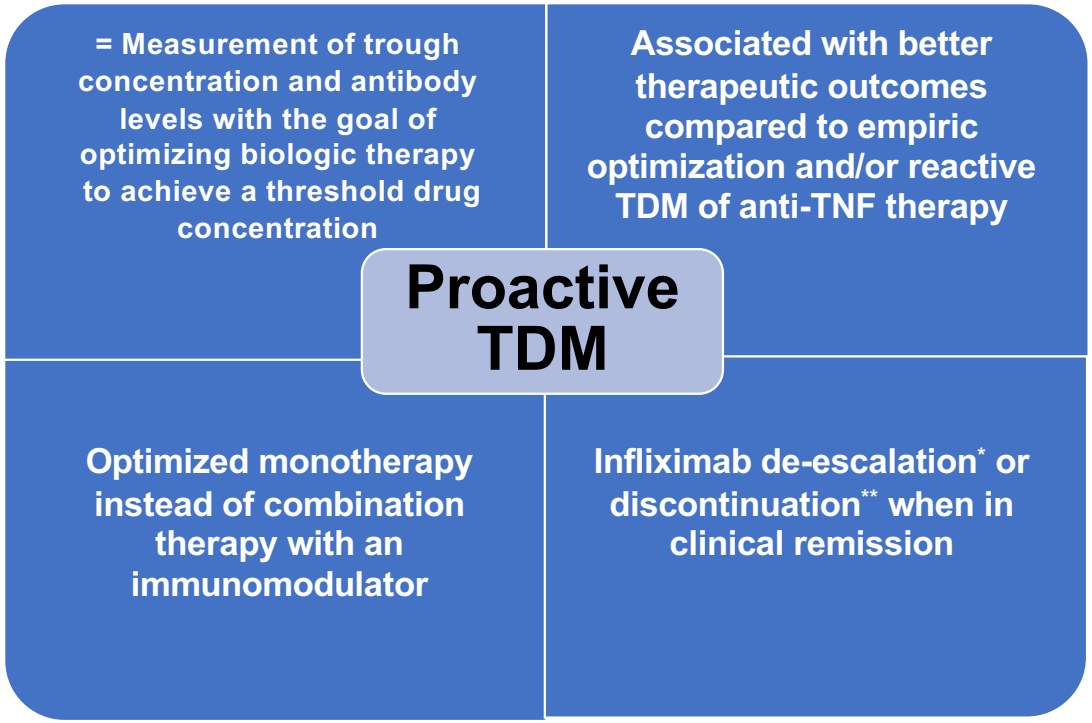
Higher drug concentrations are associated with better outcomes

Undetectable / low drug concentrations are associated with loss of response and antibodies

Box plots (5-95%) show the median (solid line within box), interquartile range (upper and lower box boundaries) and standard deviation (whiskers).

Papamichael et al 2019 CGH

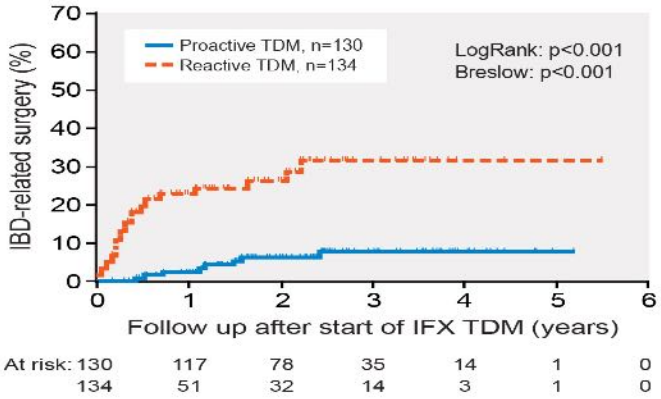
Proactive TDM of Biologics in IBD: improved response rates and prevention of ADA



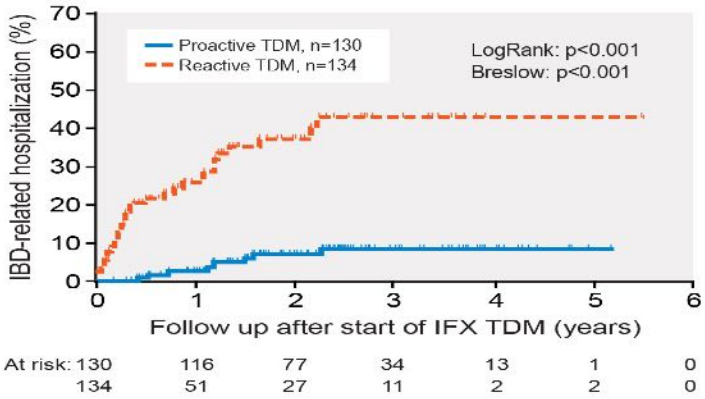
Adapted from Papamichael and Cheifetz Curr Opin Rheumatol 2020;32:371-379

Markedly Diminished IBD-related surgery, hospitalization, *anti-drug antibodies, and Serious Infusion Reactions* with Proactive TDM

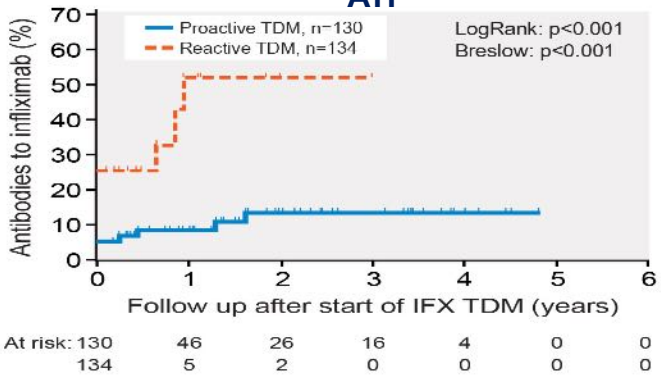
IBD-related surgery



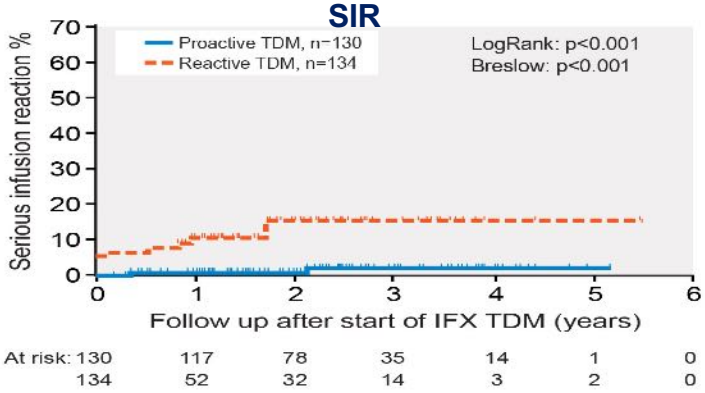
IBD-related hospitalization



ATI



SIR



- Multicenter (BIDMC and UPenn), retrospective, observational study.
- 264 patients with IBD who responded to infliximab and received maintenance therapy and underwent either proactive or reactive TDM, based on the *first* infliximab concentration / antibodies to infliximab (ATI) measurement

Papamichael K, et al. *Clin Gastroenterol Hepatol*. 2017;15(10):1580-1588.

What is the Mechanism by which Proactive TDM Reduces ADA (ATI) and Likely Induces Tolerance?

EpiVax

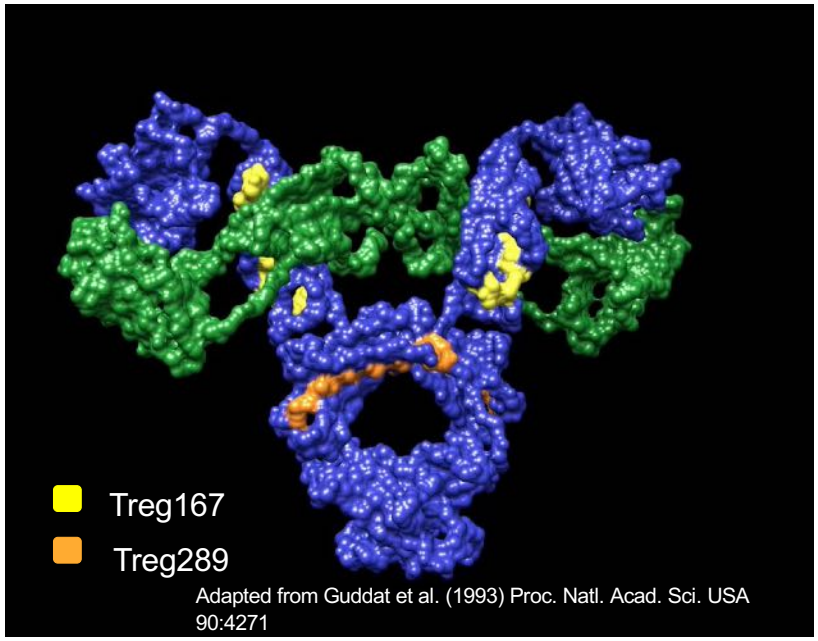
Hypothesis: By ensuring high maintained levels of TNF-mAbs immunogenicity is prevented because sustained presentation of Tregulatory epitopes present in TNF mAbs prevents or counters the immunogenic Teff epitopes

- Adalimumab: contains Tregitopes 289 and 167 in Heavy Chain
- Etanercept (Enbrel) contains Tregitope 289 in its Fc domain
- Infliximab contains Tregitopes 289 and 167

Experiments to test hypothesis:

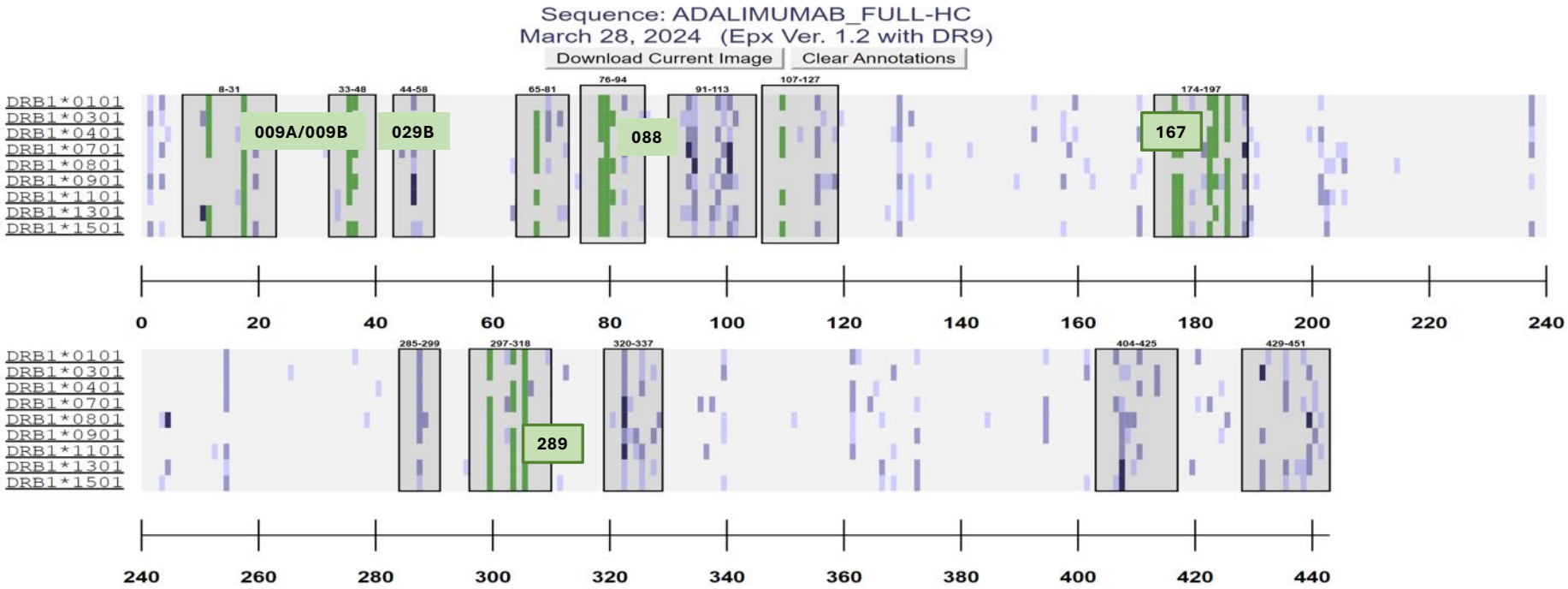
- Further information from IEDB database: functional readouts from peptide sequences defined as immunodominant and recognized as Tregitopes 167 and 289 in TNF mAbs
- MAPPS assay verification using B cell populations, macrophages, DCs to evaluate presentation of Tregitopes embedded in TNF-mAbs on HLA class I and II.
- HLA transgenic mice treated with TNF-mAbs

Regulatory T cells Epitopes (Tregitopes) Present in Immunoglobulin IgG Conserved Domains



- 15-20 aminoacid peptides in conserved IgG regions
- Tregitopes are presented by multiple HLA Class II molecules
- Highly conserved among IgG molecules across species: potential basis for IVIG induced regulation
- Induce natural Tregs to modify immune responses

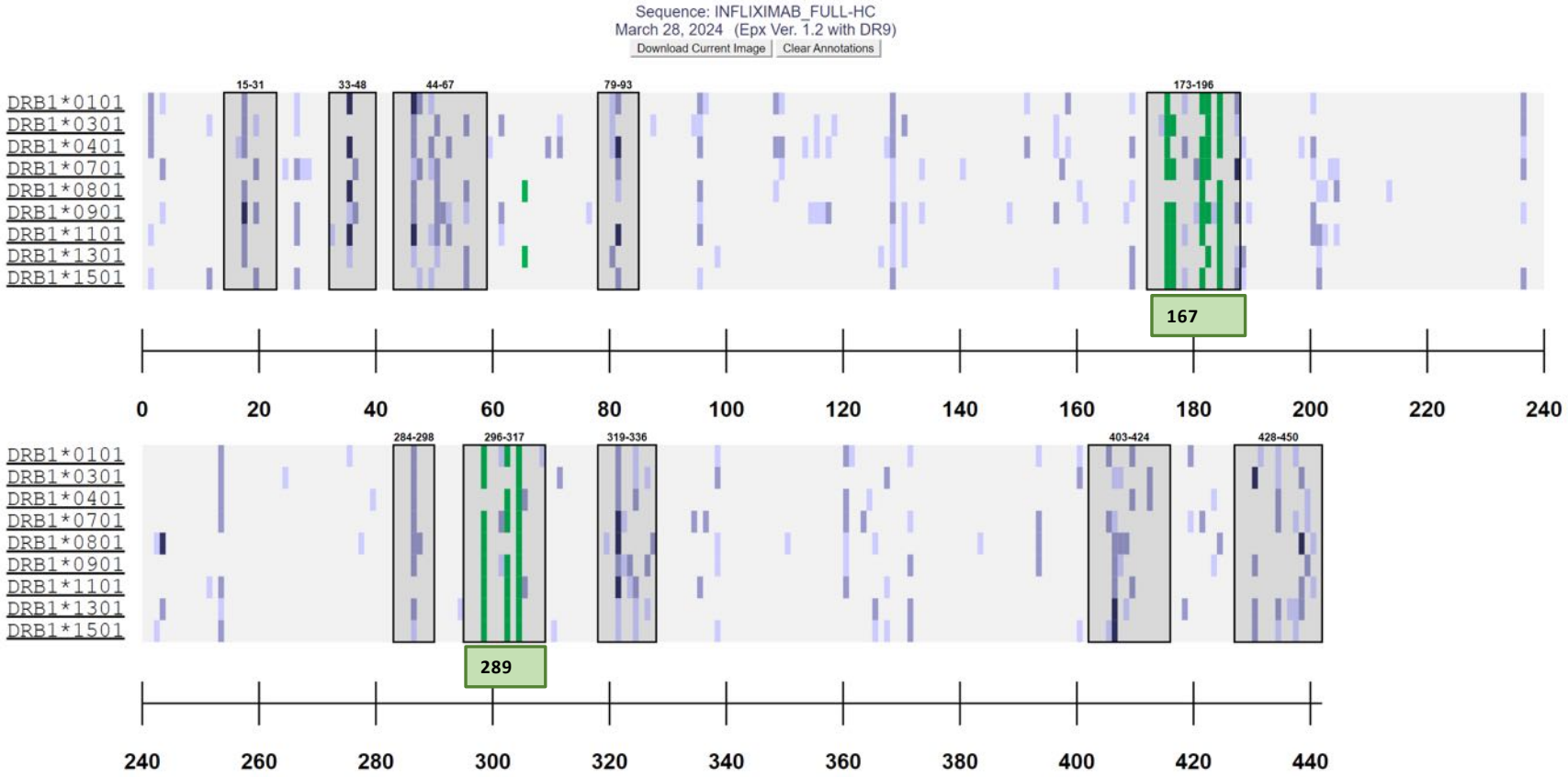
Tregitopes 167 and 289 in Adalimumab Heavy Chain



Summarized Results	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*0901	DRB1*1101	DRB1*1301	DRB1*1501	Total
Maximum Single Z-score	3.30	2.50	3.41	2.90	2.73	3.06	3.43	2.91	2.82	--
Sum of Significant Z-scores	62.49	55.32	68.09	67.49	46.80	67.27	46.93	45.20	44.06	503.65
Count of Significant Z-scores	32	29	33	34	22	32	23	23	22	250

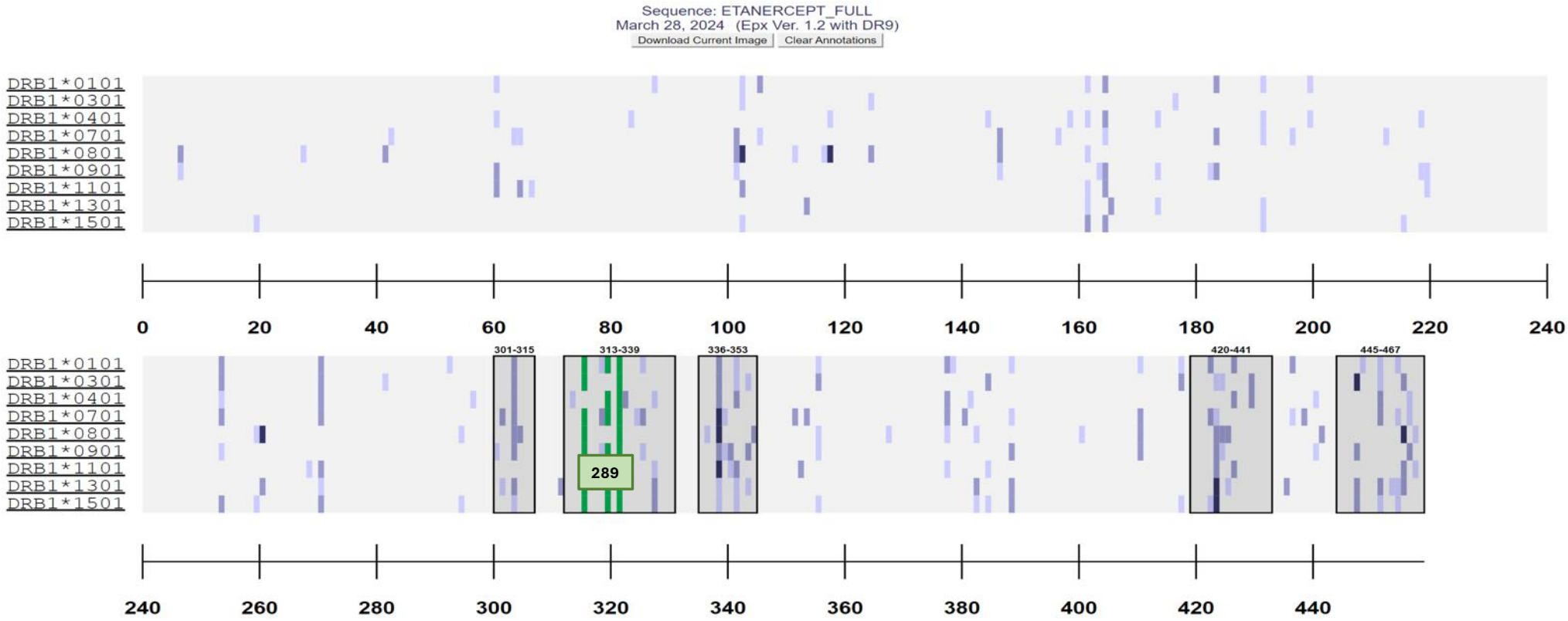
Total Assessments Performed: 3987	Deviation from Expectation: 60.06	Deviation per 1000 AA: 15.06
Adjusted for Regulatory Epitopes	Deviation from Expectation: -125.67	Deviation per 1000 AA: -31.52
		JanusMatrix Score: 8.89

Tregitopes 167 and 289 in Infliximab HC



Summarized Results	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*0901	DRB1*1101	DRB1*1301	DRB1*1501	Total
Maximum Single Z-score	2.45	2.50	2.56	2.63	2.71	2.62	2.51	2.84	2.49	--
Sum of Significant Z-scores	49.39	45.71	63.65	53.19	42.89	56.56	43.27	36.56	33.93	425.15
Count of Significant Z-scores	26	24	32	27	21	28	22	19	17	216
Total Assessments Performed: 3978			Deviation from Expectation: -17.40				Deviation per 1000 AA: -4.37			
Adjusted for Regulatory Epitopes			Deviation from Expectation: -101.74				Deviation per 1000 AA: -25.58			
							JanusMatrix Score: 5.35			

Tregitope 289 in Etanercept



Summarized Results	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*0901	DRB1*1101	DRB1*1301	DRB1*1501	Total
Maximum Single Z-score	2.18	2.50	2.42	2.63	2.78	2.43	2.41	2.84	2.49	--
Sum of Significant Z-scores	25.58	21.21	24.12	35.28	41.79	29.75	26.85	28.84	15.45	248.87
Count of Significant Z-scores	14	11	12	19	20	15	14	15	8	128
Total Assessments Performed: 4131	Deviation from Expectation: -210.68						Deviation per 1000 AA: -51.00			
Adjusted for Regulatory Epitopes	Deviation from Expectation: -243.41						Deviation per 1000 AA: -58.92			
							JanusMatrix Score: 4.90			

What is the Mechanism by which Proactive TDM Reduces ADA and may Induce Tolerance?

EpiVax

Experiments to test hypothesis:

- Further information from IEDB database: functional readouts from peptide sequences defined as immunodominant and recognized as Tregitopes 167 and 289 in TNF mAbs
- MAPPS assay verification using B cell populations, macrophages, DCs to evaluate presentation of Tregitopes embedded in TNF-mAbs on HLA class II.
- HLA transgenic mice treated with TNF-mAbs

Acknowledgements

- Aimee Mattei, Andres Gutierrez, Riley Nolan and Anne De Groot, EpiVax
- Adam Cheifetz, BIDMC
- Priya Kishnani, Duke Department of Pediatrics
- Jeff Bluestone, Sonoma Biotherapeutics
- Kevan Herold, Yale School of Medicine

EpiVax