Risk Assessment and Mitigation of Immune Responses to Therapeutic Proteins: Immune Tolerance Induction and Protein Engineering

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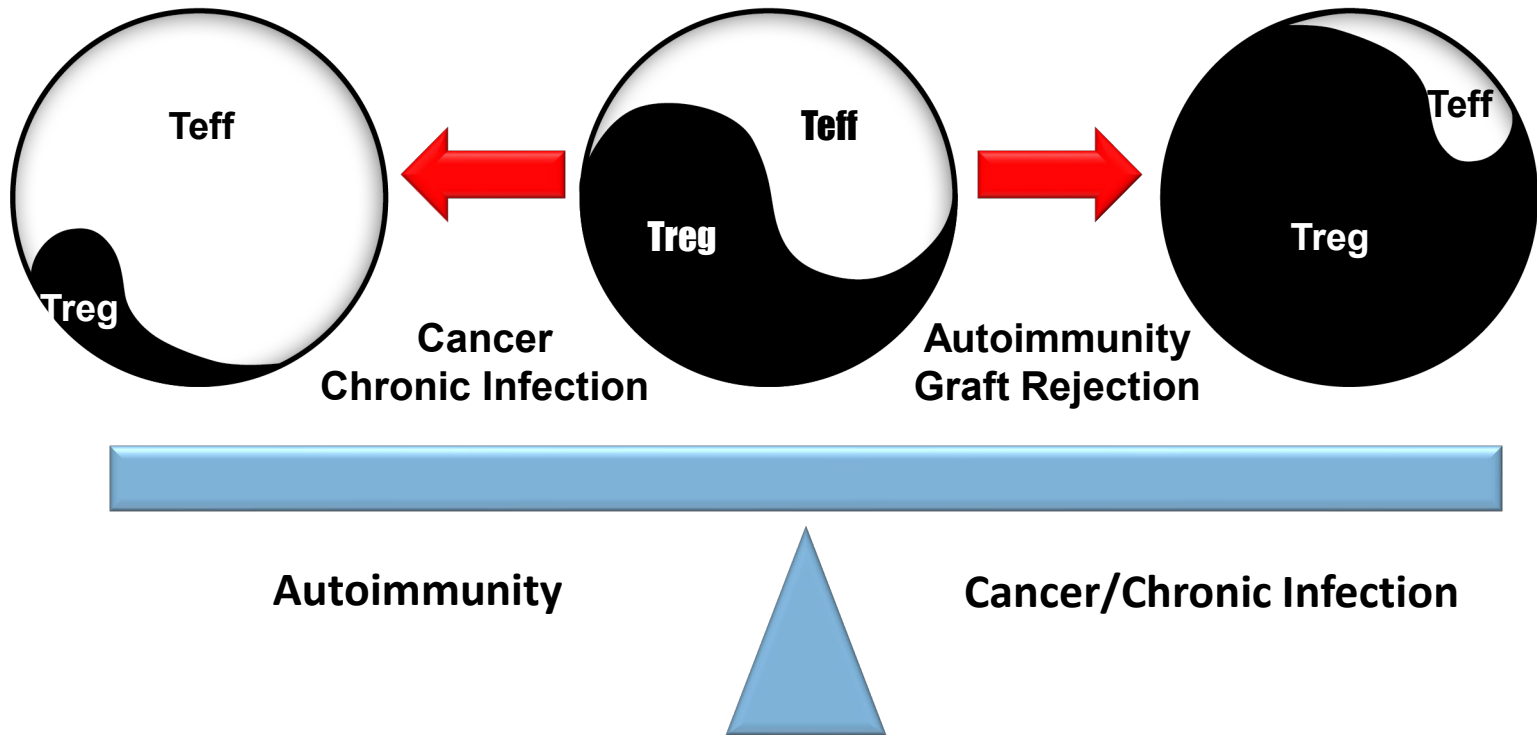Supervisory Medical Officer and Division Director, Office of Biotechnology Products

CDER, FDA

Disclosure and Disclaimer

Unless specified as FDA Guidance or Regulations, this speech reflects the views of the author and should not be construed to represent FDA's views or policies. I have no financial relationships to disclose.

The Immunology Revolution:
Tipping the Balance for Therapy of Complex Diseases

Teff

Treg

Cancer
Chronic Infection

Teff

Treg

Autoimmunity
Graft Rejection

Teff

Treg

Autoimmunity

Cancer/Chronic Infection

"Guidance for Industry Immunogenicity Assessment for Therapeutic Protein Products"

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

August 2014
Clinical/Medical

Immunogenicity of Therapeutic Proteins Risk Assessment: Consequences for Safety

- Fatality/Severe Morbidity
  - Anaphylaxis-clinical definition, does not imply mechanism
    - Proteins of non-human origin, eg, aprotinin, asparaginase
    - Replacement human proteins in knock out phenotype: eg, Factor IX in hemophilia B
    - Cytokine release syndrome; mAbs that target and cluster cell surface receptors; not immunogenicity per se
  - Cross reactive antibody mediated neutralization of 1) endogenous factor with non-redundant function resulting in a deficiency syndrome or 2) receptor homolog resulting in a cytokine release syndrome
  - Immune Complex Mediated Disease: delayed hypersensitivity
    - Serum sickness; nephropathy
    - Observed in the context of immune tolerance induction when high doses of therapeutic protein administered in setting of robust antibody response

Immunogenicity Risk Assessment Consequences for Efficacy

- Neutralizing antibodies to life saving therapeutics
  - eg., Enzyme and Coagulation Factor Replacement Therapies

- Diminished efficacy of highly effective therapeutics
  - mAbs: eg TNF blockers

- Alterations in PK
  - Antibodies to protein therapeutics may diminish or enhance PK/PD
    - Sustained or increased response may lead to epitope spread, generation of neutralizing antibodies, Type III hypersensitivity responses (circulating immune complexes)
    - Changes in dosing level and schedule (ie "dosing over") may generate CIC

- No apparent effect
  - But sustained response may lead to epitope spread and generation of neutralizing responses, eg. IL-2

Immunogenicity Risk Assessment: Patient and Protocol Factors

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Clinical Characteristics

Dosing Frequency	Single	Chronic	Intermittent
Dose Concentration	Very High		Low-Average
Route of Administration	Oral	i.v. s.c.	Inhaled
Patient Immune Status	Suppressed	Healthy	Activated
Immunomodulatory Action	Immunosuppressant		Immunostimulant
Endogenous Protein Level	High		Low

PROBABILITY =   **LOW**   **UNKNOWN**   **HIGH**

Modified by Barry Cherney and Amy Rosenberg from Holly W. Smith, Eli Lilly

Immunogenicity Risk Assessment

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IMPACT =	**LESS SERIOUS**		**SEVERE AE**

Endogenous homolog?	No		Yes
Redundant/Unique Biology?	Redundant		Unique
Impact of Autoimmune/KO	Minimal	Manageable	Fatal
Intended Disease IND	Not Life-threatening		Life-threatening
Intended Disease Post AP	Not Life-threatening		Life-threatening
Treatment Options	Other options available	Other options not available	

Modified by Barry Cherney and Amy Rosenberg from Holly W. Smith, Eli Lilly

Acting on Immunogenicity Risk Assessment: Consequences Determine Action

- When consequences are life threatening, immune tolerance induction may be life saving
  - "immune tolerance is broadly defined as a selective elimination of pathogenic immune responses to relevant antigens (e.g. autoantigens) by any of a variety of approaches (deletion, induction of anergy, immune deviation, sequestration, or suppression) while preserving protective immunity *and does not require ongoing treatment with the intervention.*"
  - Immune tolerance induction should also be considered when the immune response abolishes efficacy of highly effective (but not necessarily life saving) therapeutics: eg TNF antagonists
    - Risks associated with tolerance regimens and impact of tolerance regimen on underlying disease course should be considered

- Deimmunization of a protein therapeutic an appropriate approach in non-urgent clinical scenarios
  - Use of predictive algorithms and in vitro studies to identify and remove immunogenic epitopes
    - protein engineering should ensure that other critical attributes of the therapeutic protein are not altered for the worse such as activity, aggregation, deamidation etc
    - Approach should be considered at early stages of product development and especially for high risk proteins in specific patient populations
    - Evaluation in appropriate spectrum of human HLAs essential

Modalities of Tolerance Induction: Antigen Specific

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- Antigen- the bait/lure; only the relevant lymphocyte populations, the catch
  - allergenics,
  - tolerizing vaccines,
  - oral tolerance approaches
  - antigen loaded nanoparticles
  - antigen specific Treg cells
  - donor cellular infusions in transplantation
- Safety concerns: potential for in vitro or in vivo conditions to convert tolerance induction to immunization
  - robust inflammation;
  - route of administration effects: eg accidental infiltration of SC space from IV delivery
  - presence of innate immune response modifiers in product or at site: DAMPs/PAMPs etc
  - **product stability within the bead formulation: aggregates, deamidation, oxidation etc**;
  - stability of Tregulatory cells (TSDR:Th17 conversion);

  Caveat: Time course for establishment of tolerance may be outside the zone of clinical necessity

Modalities of Tolerance Induction: Antigen Targeted

- Therapeutic targets lymphocyte populations broadly but specific antigen(s) targeted by close proximity of tolerance inducing therapeutic in time and location with immunologically relevant cells and target antigens
  - low dose/antibody complexed IL-2: generate/stimulate Tregs eg muscle Tregs in DMD
  - non-FcR binding CD3 mAbs: induction of T cell exhaustion in T cells mediating islet specific autoimmune responses
  - rapamycin loaded nanoparticles: tolerogenic DC generation
  - polyclonal Tregs
  - Low dose methotrexate: preliminary evidence in CRIM+ Pompe (Kazi Z et al. Genetics in Medicine (2018)
- Safety concerns:
  - global immune suppression possible;
  - tolerance induction to pathogens?
  - effects on tumor immunity?
- Efficacy Challenge: failure of untargeted therapeutic to achieve sufficient dose in relevant tissues and timeframe required for efficacy

Modalities of Tolerance Induction

- Global immune suppression: immune system regenerates under tolerogenic conditions/exposure to therapeutic
  - Autologous hematopoietic stem cell transplant for MS, Scleroderma: diseases with high morbidity and mortality and numerous as well as unknown antigenic specificities
  - Alemtuzumab
  - Cocktails of immune suppressive agents for preventive or therapeutic tolerance induction eg rituximab, methotrexate, proteasome inhibitors
  - May be necessary in the setting of life threatening ADA

- Safety concerns: severe adverse events pertaining to infectious and malignant complications

High Titer Antibody Response, not CRIM Status Per Se, Confers Negative Clinical Outcome in ERT-Treated Patients with Pompe Disease

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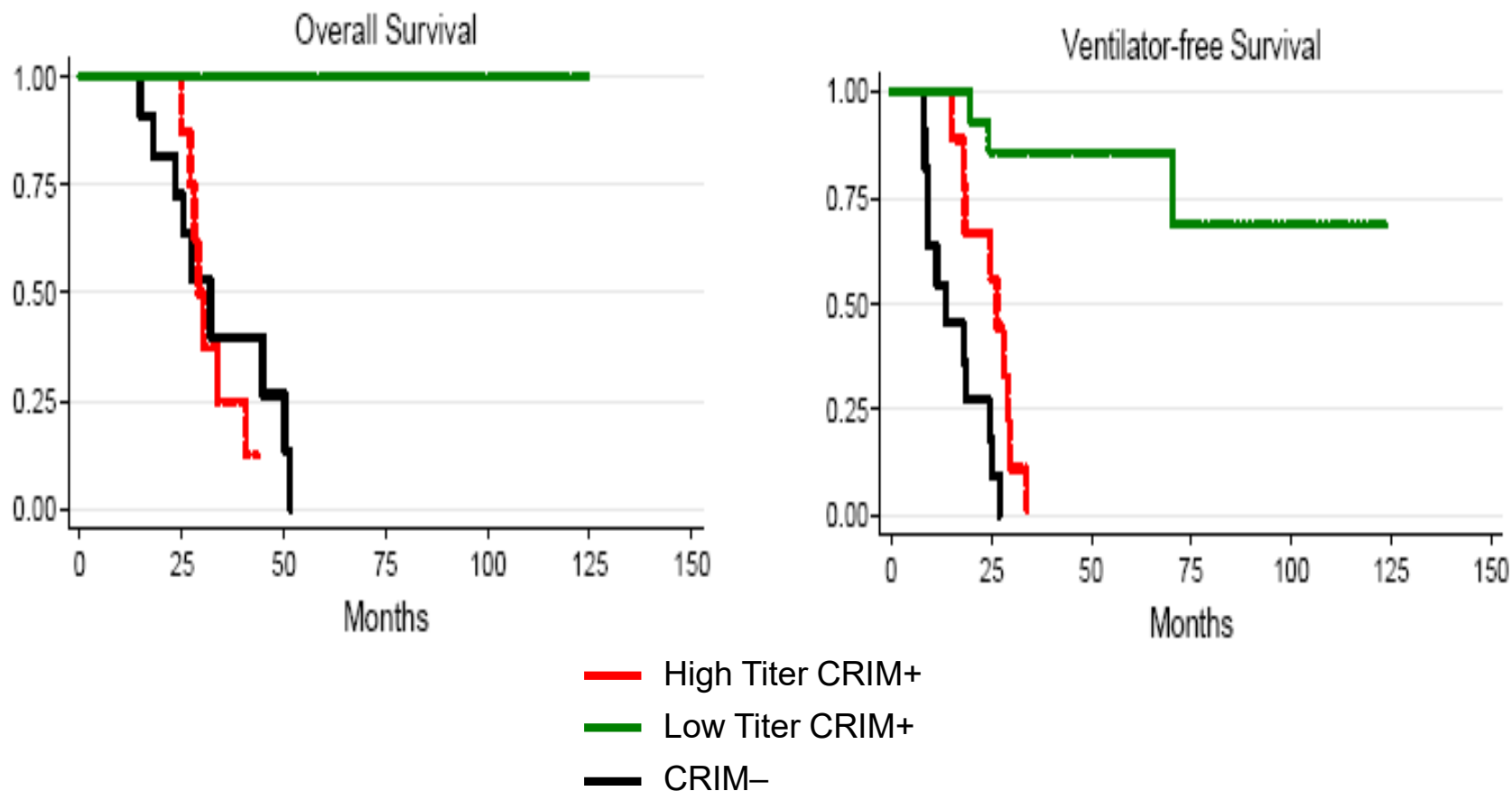
Prophylactic ITI Protocol

Banugaria S et al PlosOne 2013
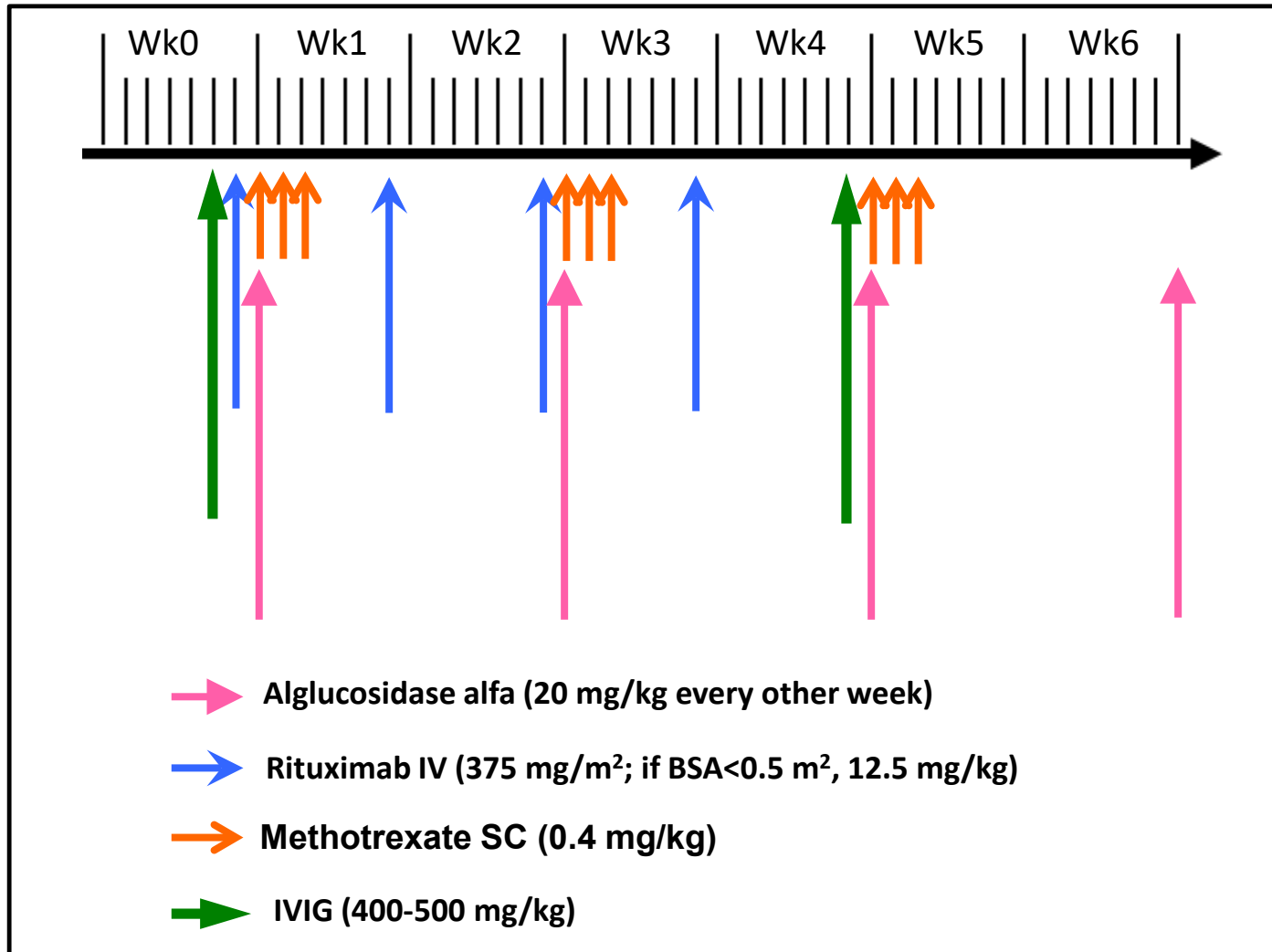
rhGAA Antibody Titer in CRIM-negative IPD Patients Treated Prophylacticallywith ERT+ITI versus ERT Monotherapy

(Kazi ZB et al JCI Insight 2017)
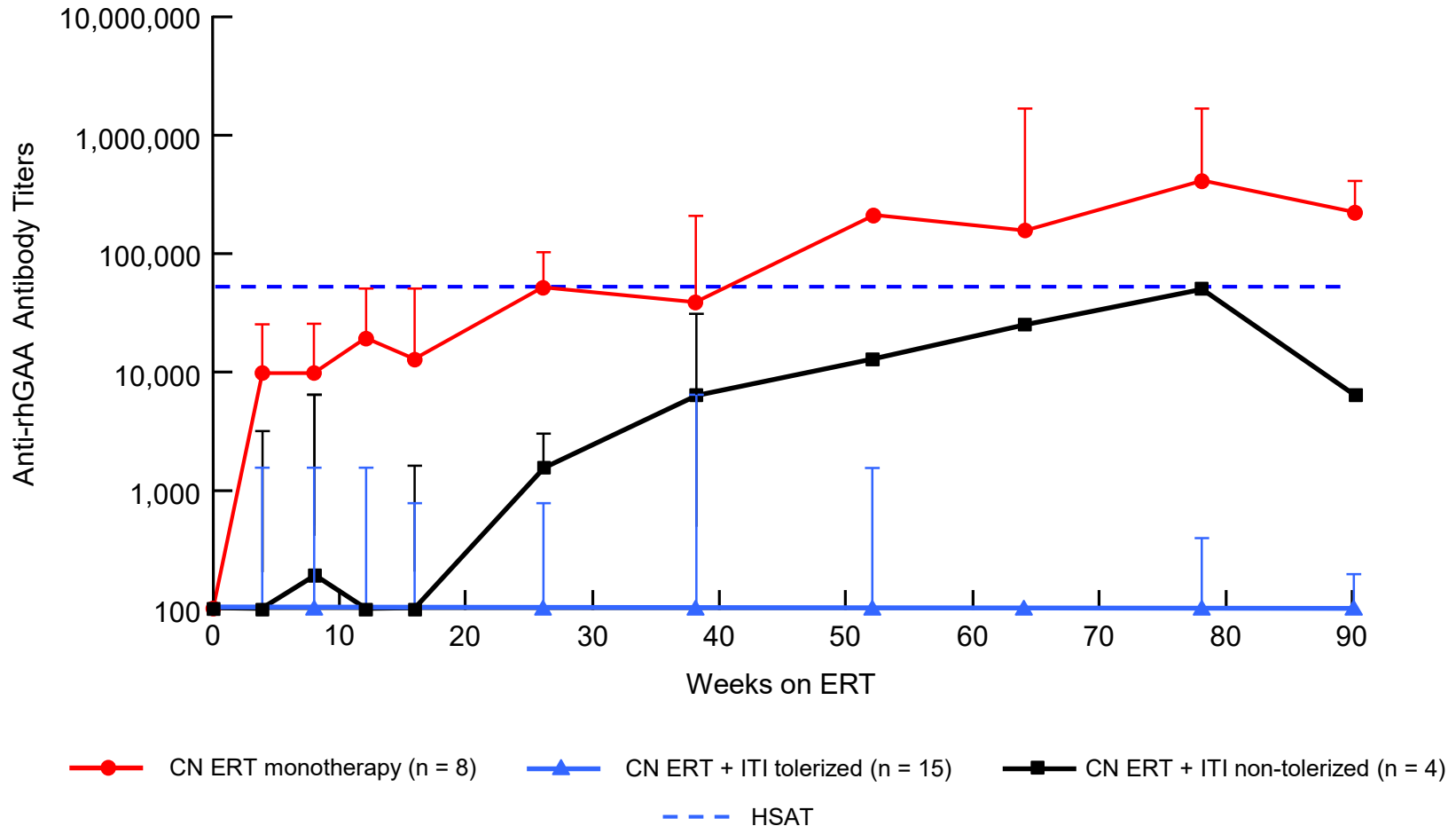
Survival of CRIM-negative IPD Patients Treated Prophylactically with ERT+ITI versus ERT Monotherapy

(Kazi ZB et al JCI Insight 2017)
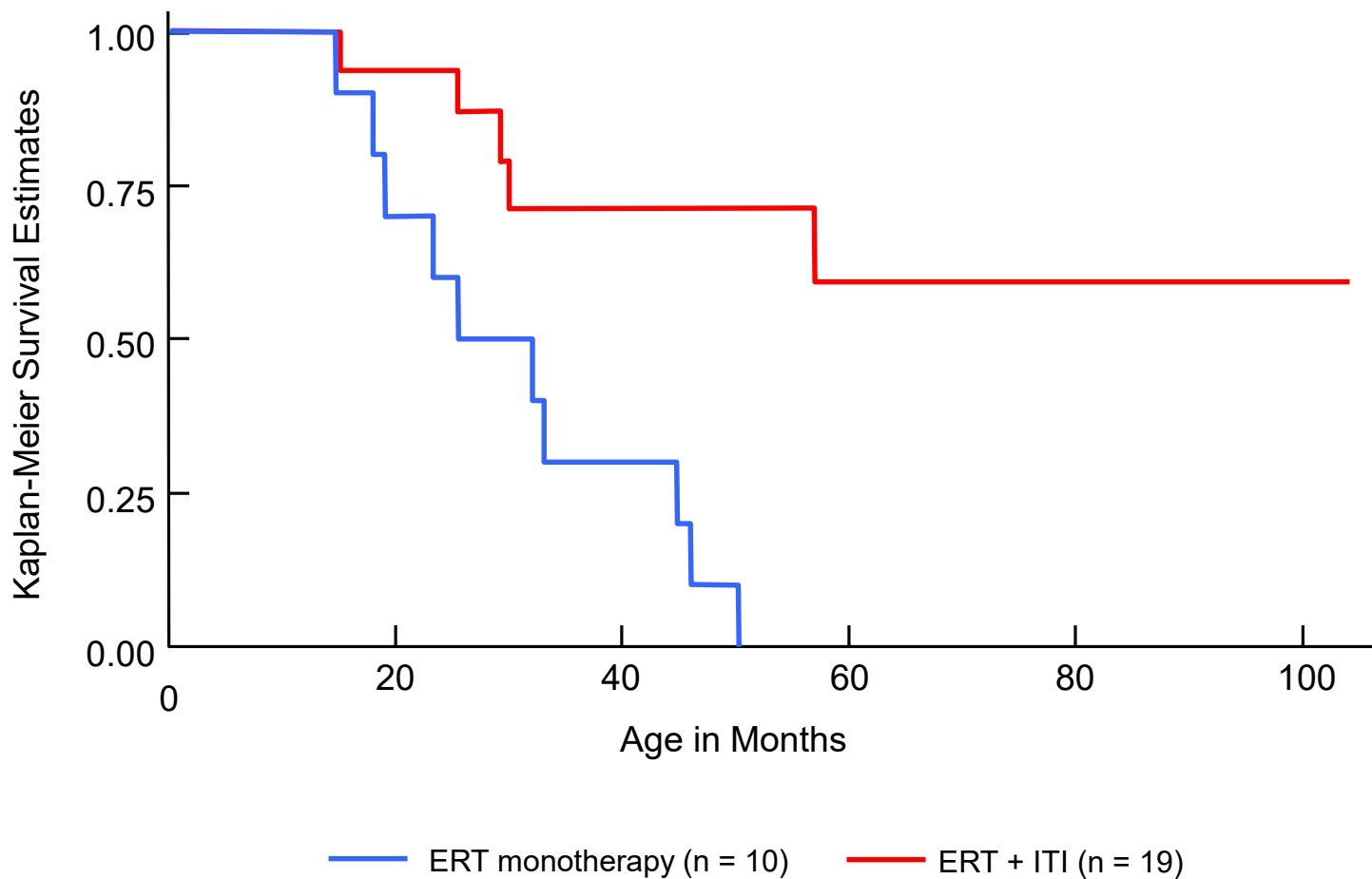
What is the Mechanism(s) of Immune Tolerance?

- Active Immune Tolerance
  - Tregs
  - Bregs
- Anergy
- Deletion

Lessons Learned from Immune Tolerance Induction to ERT for LSDs that Can Potentially Apply to Immune Tolerance to Biologics and Autoimmune Diseases

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Potential Applications for Short Course Prophylactic Tolerance Induction Strategy

- Prevention of immune responses to therapeutic proteins in patients with autoimmune conditions
  - TNF inhibitory mAbs: frequent development of antibodies that neutralize efficacy of TNF inhibitors
- Prevention of immune responses to enzyme replacement therapy in patients with other lyosomal storage diseases
  - lysosomal storage diseases in which antibodies are prominent, but clinical effect of ADA not known or investigated because clinical endpoints take years-decades to develop: preponderance of data from multiple sources indicate antibody mediated interference in treatment of Fabry Disease and MPS1

Clinical Benefit from Concomitant Immune Suppression Diminished Antibody Response to Infliximab and *Steroid Sparing*: Effect on Primary Mechanism of Disease?

(Colombel J-F et al NEJM 2010)

A Corticosteroid-free Clinical Remission at Wk 26
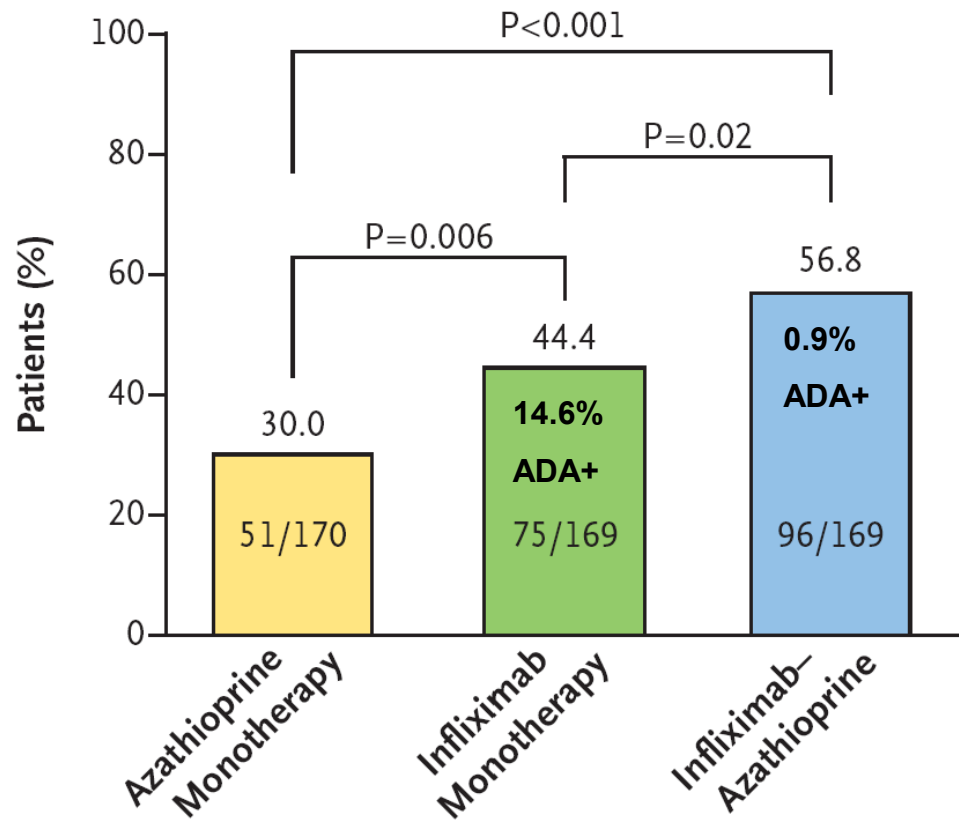
Diminished Immunogenicity/Enhanced Efficacy of Concomitant Immunosuppressive Treatment in Autoimmune Disease: Is there a Downside?

- No difference in rate of serious infections in many studies: eg 4-5% in all groups (Colombel et al 2010). ***Requirement for steroid pulses heightens infectious risk***.

- Are patients who receive concomitant immunesuppression, especially MTX, immune tolerant to TNF mAbs? Treg population specific for mAbs?

- Would short course of tolerance inducing agents (CD20 mAb, MTX, IVIG) at onset of mAb therapy induce tolerance to therapeutic per experience with Pompe? Could this regimen also address immune pathology underlying autoimmunity?

- Combination of azathioprine and anti-TNF biologic agents increases the relative risk of hepatosplenic T-cell lymphoma. Identifiable subset of patients at higher risk.

Entrenched Antibody Responses: Unresponsive to Immune Suppressive Agents
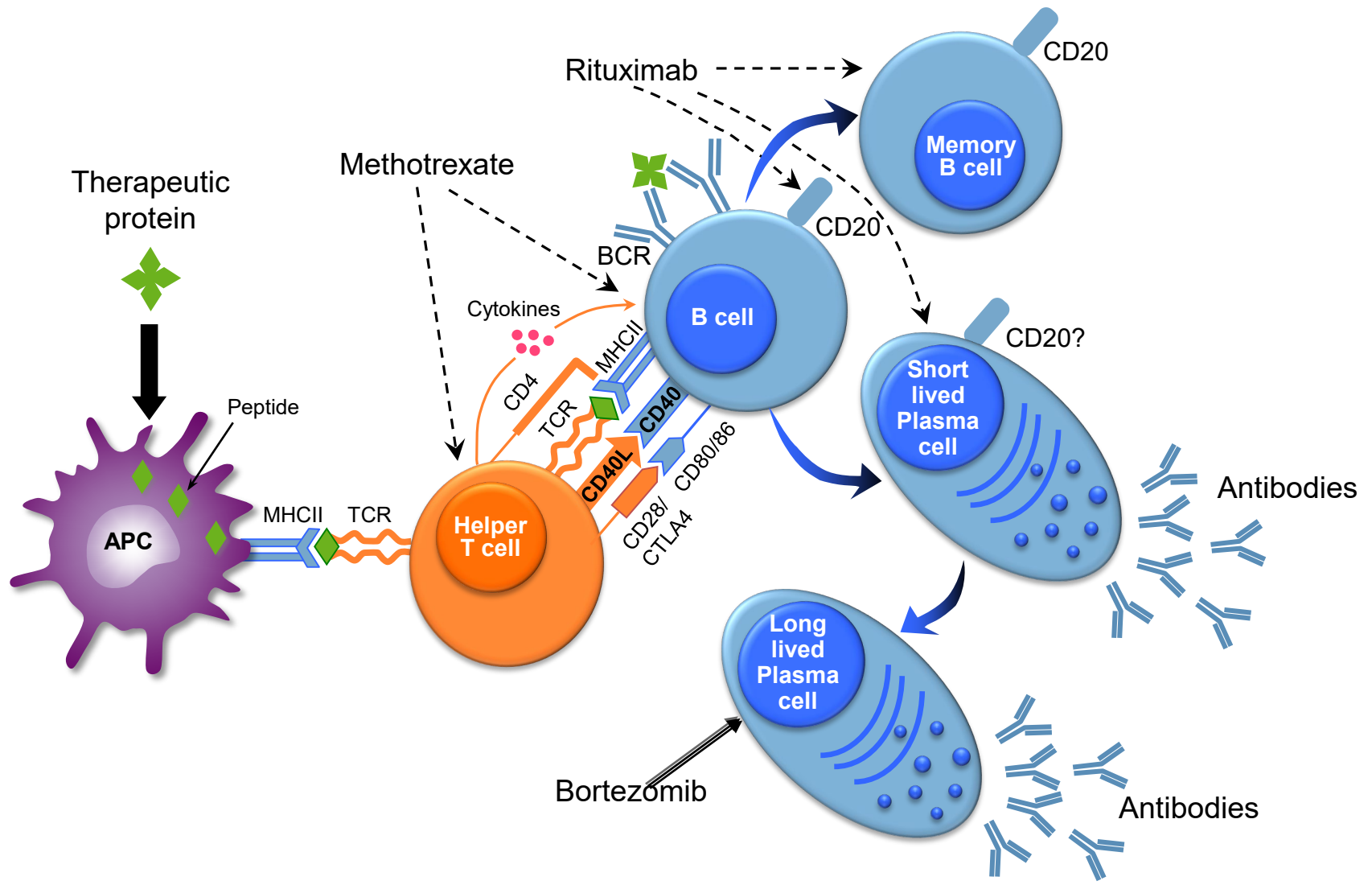(PS Kishnani et al 2012)
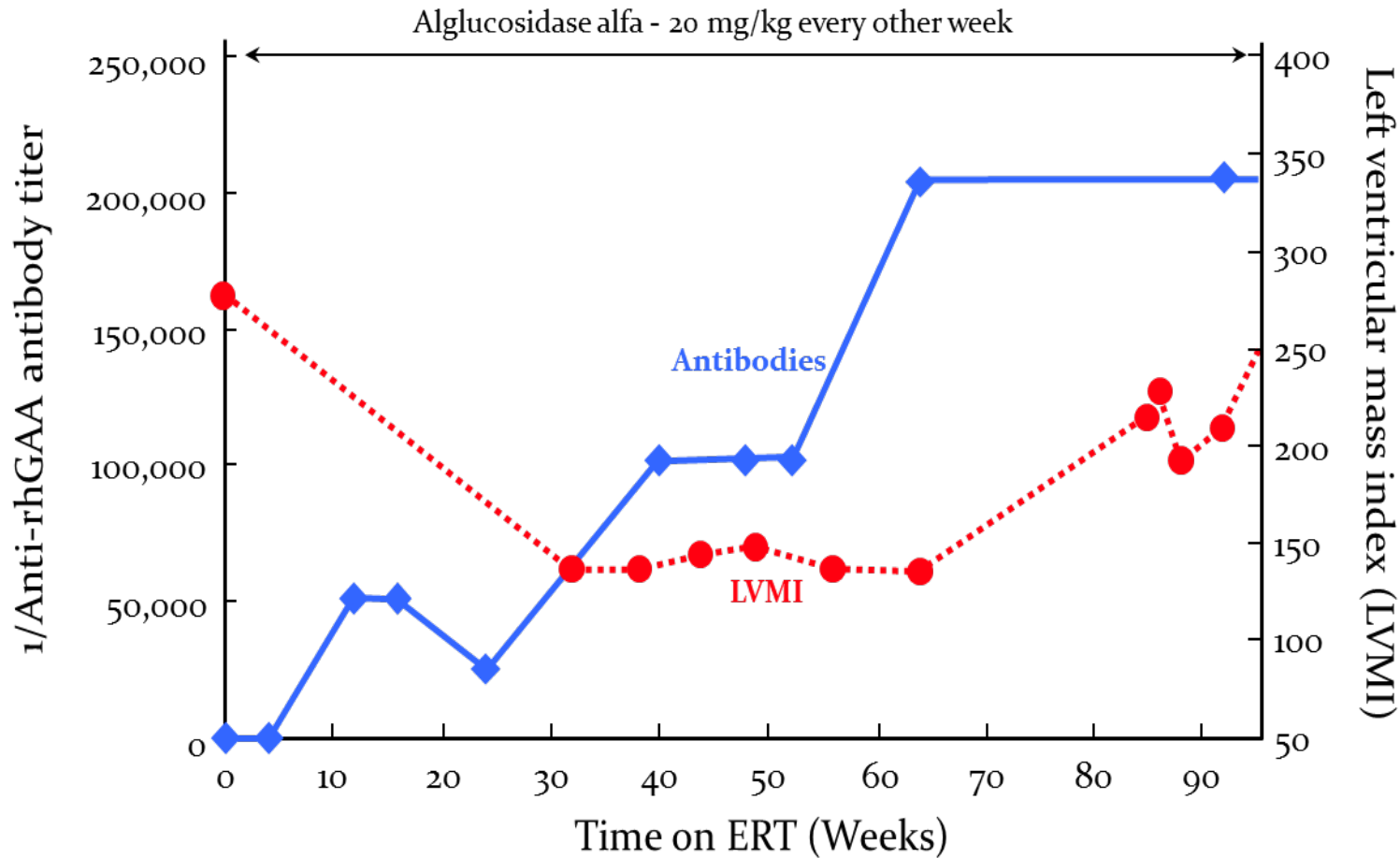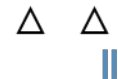
High Sustained Antibody Responses are Mediated by Long Lived Plasma Cells Unaffected by MTX/Rituximab

Targeting Long Lived Plasma Cells
with Bortezomib Dramatically Reduces Antibody Titer in Patients with HSAT

(Kishnani PS et al 2012)

Sustained Immune Tolerance to ERT Following Discontinuation of Immune Suppressives in Patients with High Sustained ERT Antibodies

Kazi ZB et al JCI Insight 2016
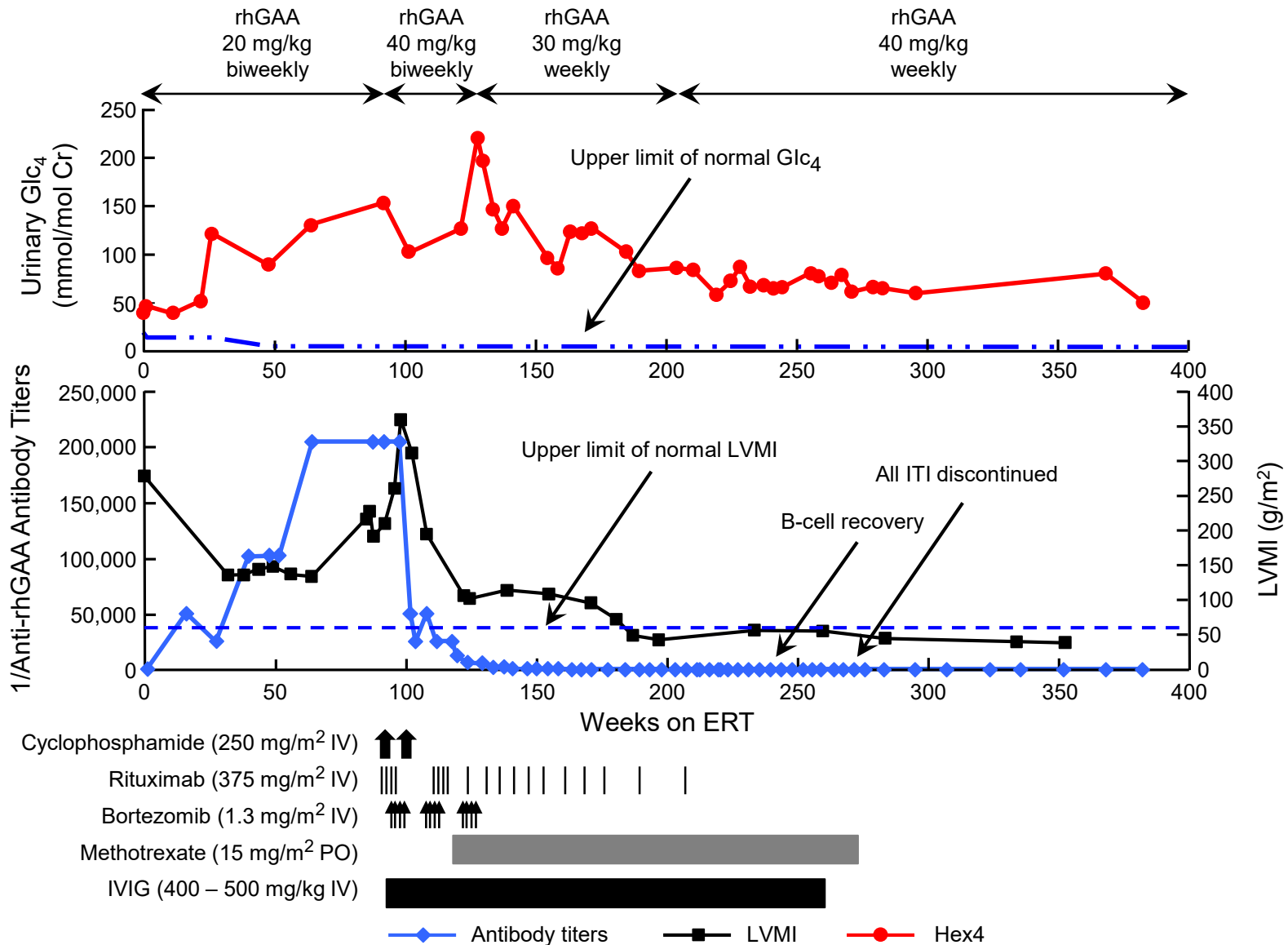
What is the Mechanism of Immune Tolerance?

- Active Immune Tolerance
  - Tregs
  - Bregs
- Anergy
- Deletion

Autoimmune Diseases with Pathogenic Autoantibodies: Can Targeting Long Lived Plasma Cells Improve Clinical Outcome?

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Diseases with pathogenic antibodies	Antibodies
SLE	Anti-DNA, anti-RNP
RA	RF anti-CCP
Vasculitis	Anti-myeloperoxidase, anti-proteinase 3
Myasthenia gravis	Anti-acetylcholine receptor
Hypothyroidism	Anti-thyroglobulin
Hyperthyroidism	Anti-TSH receptor
Vitiligo	Anti-melanocytes (melanin concentrating hormone receptor (MCHR1)
Pernicious anemia	Anti-intrinsic factor, anti-parietal cell
Neuromyelitis optica	Anti-aquaporin 4, anti-MOG
Addison's disease	Anti-cytochrome p450
Primary biliary cirrhosis	Anti-pyruvate dehydrogenase
Pulmonary Alveolar Proteinosis	Anti-GMCSF
Limbic encephalitis	Anti-GluN1 of the NMDA receptor
Pemphigus	Anti-desmoglein
Celiac disease	Anti-transglutaminase
Anti-phospholipid syndrome	Anti-cardiolipin, anti-β2GP1
Hemolytic anemia	Anti-RBC
ITP	Anti-platelet
Anti-Synthetase/Dermatomyositis	*Anti: syn, DM, necrotizing myop associated*

Proteasome Inhibition in a Patient with SLE Related Myocarditis and Nephritis: dsDNA a Known Target Antigen but Utmost Clinical Urgency
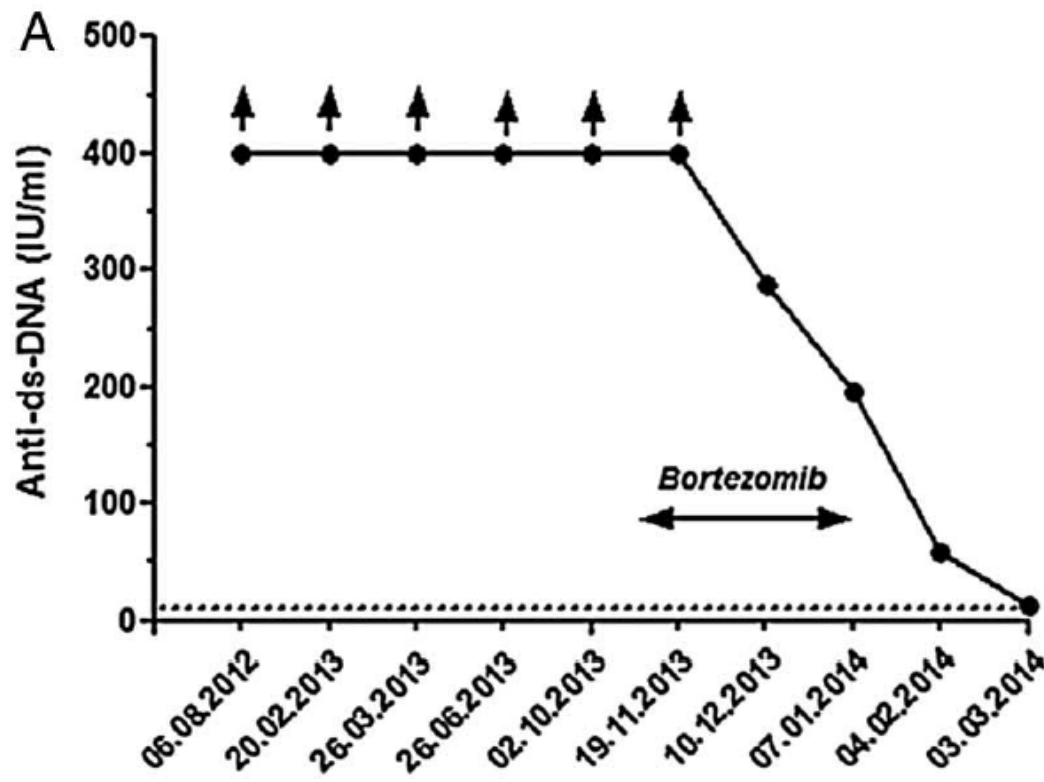
Safety Issues Associated with Prolonged Immune Suppression

- Vaccine responses prevented or eliminated under prolonged non-specific immune suppression

- Reactivation of latent infections including JC virus: risk of PML

- Enhanced risk of malignancies

- Prophylactic tolerance induction recommended approach:
  - much reduced duration of immune suppression
  - clear tolerance measures;
  - may prevent irreversible tissue damage due to diminished ERT activity.

Reminder: Anti Drug Antibody Responses *May* Not Always Negatively Impact Safety and Efficacy

- Neutralizing antibody may act as a "chaperone" or "carrier" for therapeutic proteins, enhancing PK and, potentially, product activity and efficacy.

- *Such "favorable" antibody responses are unpredictable, uncontrolled and may represent a metastable or unstable state: this requires intensive study*

- Suggests novel approach to formulation for prolonged activity of ERT and cytokines (eg IL-2+IL-2mAb conjugate)

Elosulfase alpha for MPS IVA (Morquio Syndrome): Are Nabs Friend or Foe?

- All patients developed anti-drug antibody by week 4. Antibody titers were sustained or increased over 72 weeks of treatment.

- By week 16, ~96% of patients developed neutralizing antibodies capable of inhibiting the drug from binding the mannose-6-phosphate receptor and being taken up into cells.

- ***However, instead of enhanced ERT clearance due to antibodies, clearance was delayed***

  - Mean AUC_{0-t} and Cmax increased to 2.8-and 2.9-fold, respectively, at Week 22 compared to Week 0.

  - Mean $t^{1/2}$ increased from 7.5 min at Week 0 to 35.9 min at Week 22.

- Suggests that in patients mounting nAB responses, antibody is acting as a chaperone/carrier bound to elosulfase

- In these cases, elosulfase may dissociate from antibodies and potentially bind to target tissues, improving activity and efficacy: is that the case?

- Since all patients developed anti-drug antibodies, available data are inadequate to assess the relationship between antibody development and therapeutic response and whether immune tolerance induction could be beneficial.

Can we Exploit Antibodies as Carriers to Enhance PK/PD?

- Products in development as complexed with mAbs as drug product, eg IL-2/IL-2mAb

- Better understanding of responses that enhance vs abrogate efficacy:
  - do such responses persist or evolve in more positive or negative directions?
  - can we learn to elicit favorable vs detrimental ADA responses?

Problem: Treatment of Refractory Cancers by Recombinant Immunotoxin Limited by Immune Response to Toxin Moiety

- Suppression of immune responses in these patients effective for delivering more treatment courses but anti-tumor responses arising from initial targeting of tumor by immunotoxin may be precluded or eliminated

- Engineer the therapeutic to maximize activity but limit immunogenicity

Recombinant immunotoxin for cancer treatment with low immunogenicity by *identification and silencing of human T-cell epitopes*

Ronit Mazor,, Jaime A. Eberlea, Xiaobo Hua, Aaron N. Vassalla, Masanori Ondaa, Richard Beersa, Elizabeth C. Leea
Robert J. Kreitmana, Byungkook Leea, David Baker, Chris King, Raffit Hassana, Itai Benharb, and Ira Pastan

Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892;

Moxetumumab Pasudotox: CD22 binding Fv+PE38 for HCL

T Cell Epitope Map of a Recombinant 2 Domain Immunotoxin
(Mazor et al 2014)
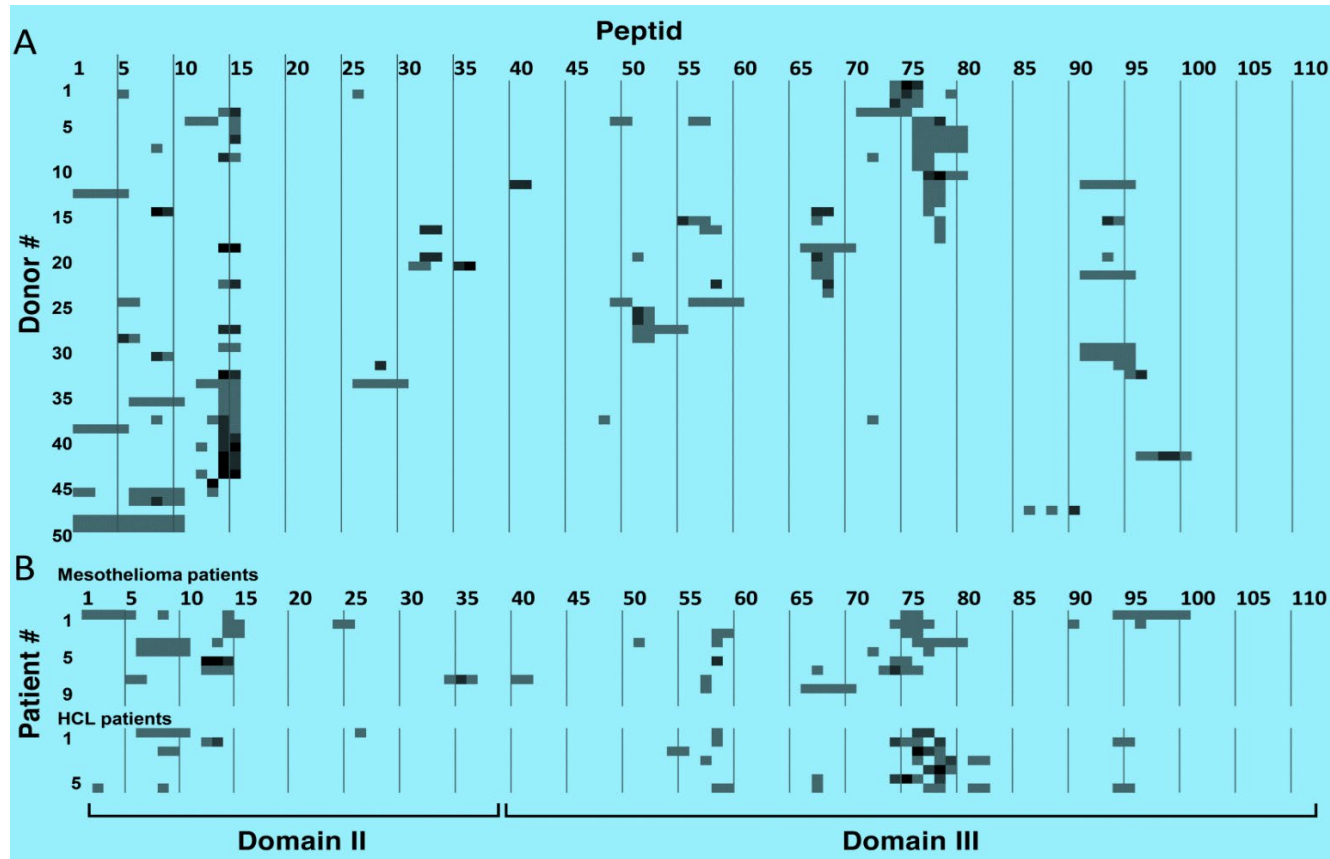
Cells were stimulated and expanded with whole RIT for 14 days and restimulated with PE38 peptides. T cell responses were measured using IL-2 ELISpot: black (>20% of spots), dark gray (10%-20% of spots), gray (3%-10% of spots) and white (<3% of spots and 80 SFC/1E6 cells)

Poor correlation between T-cell activation assays and HLA-DR binding prediction algorithms in an immunogenic fragment of Pseudomonas exotoxin A (Mazor R et al Journal of Immunological Methods: 425 (2015))

Strategy: Remove Immunogenic Domain not Critical for Activity, Introduce Mutations in T Cell "Hotspots" in Activity Critical Domain

(Mazor et al 2014)
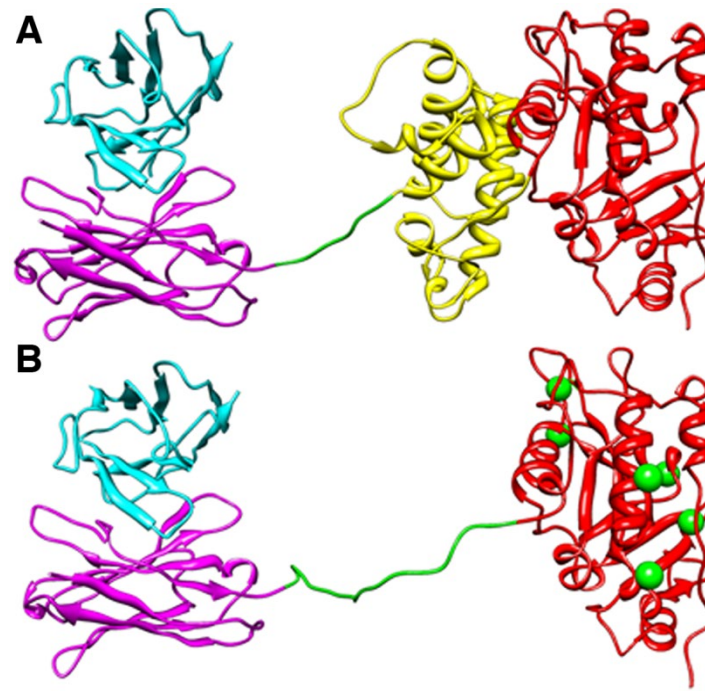
Introduction of Alanine Substitutions for Defining Critical AAs in T Cell Epitopes in Domain III
(Mazor et al 2014)
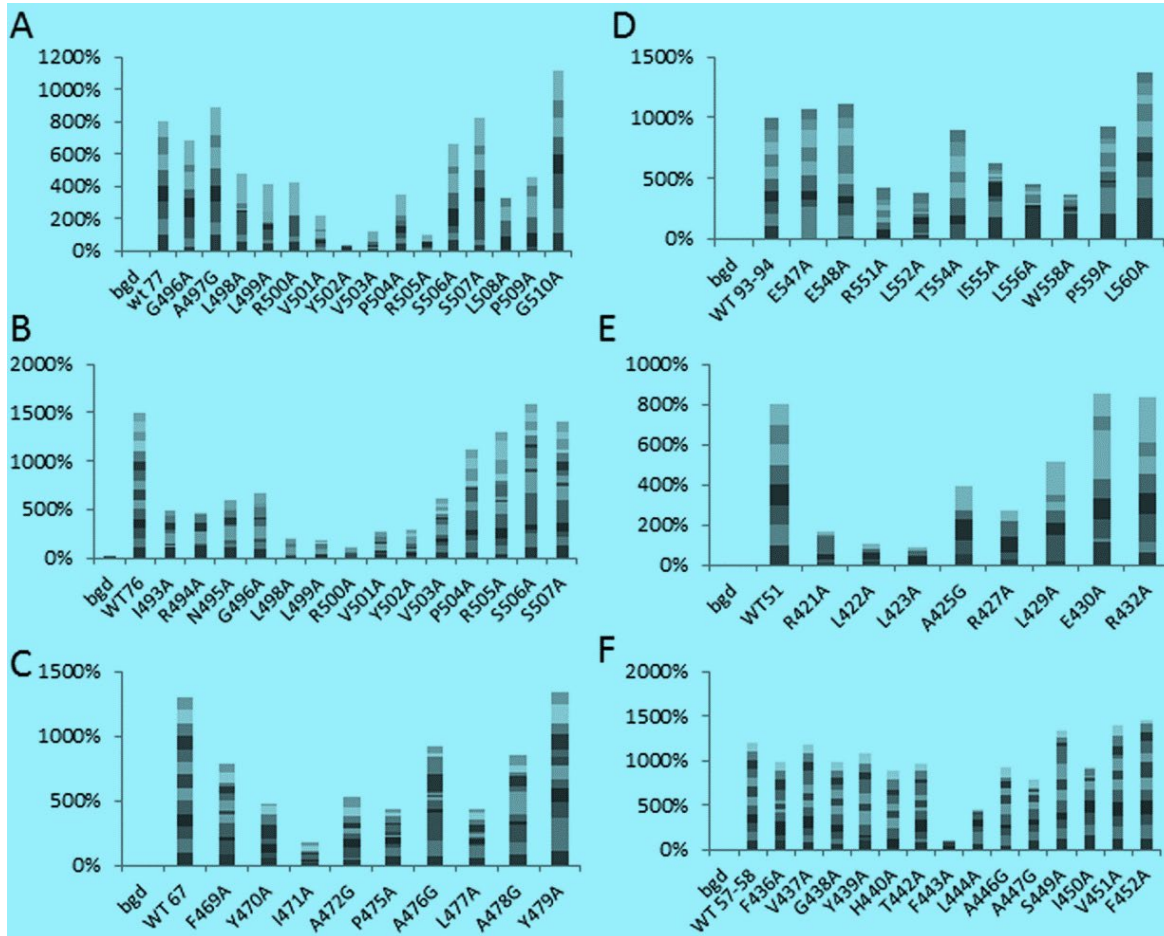
Best mutant, deimmunized RIT contained six point mutations in epitopes 2A 2B, 5,6,7 and 8

Engineered Recombinant Immunotoxin has Markedly Reduced T Cell Responses

(Mazor et al PNAS 2014)

Marked Reduction in Patient Antibody Binding to Deimmunized RIT *but Number of Treatment Courses Still Limited by Immune Response*

(Mazor et al 2014)
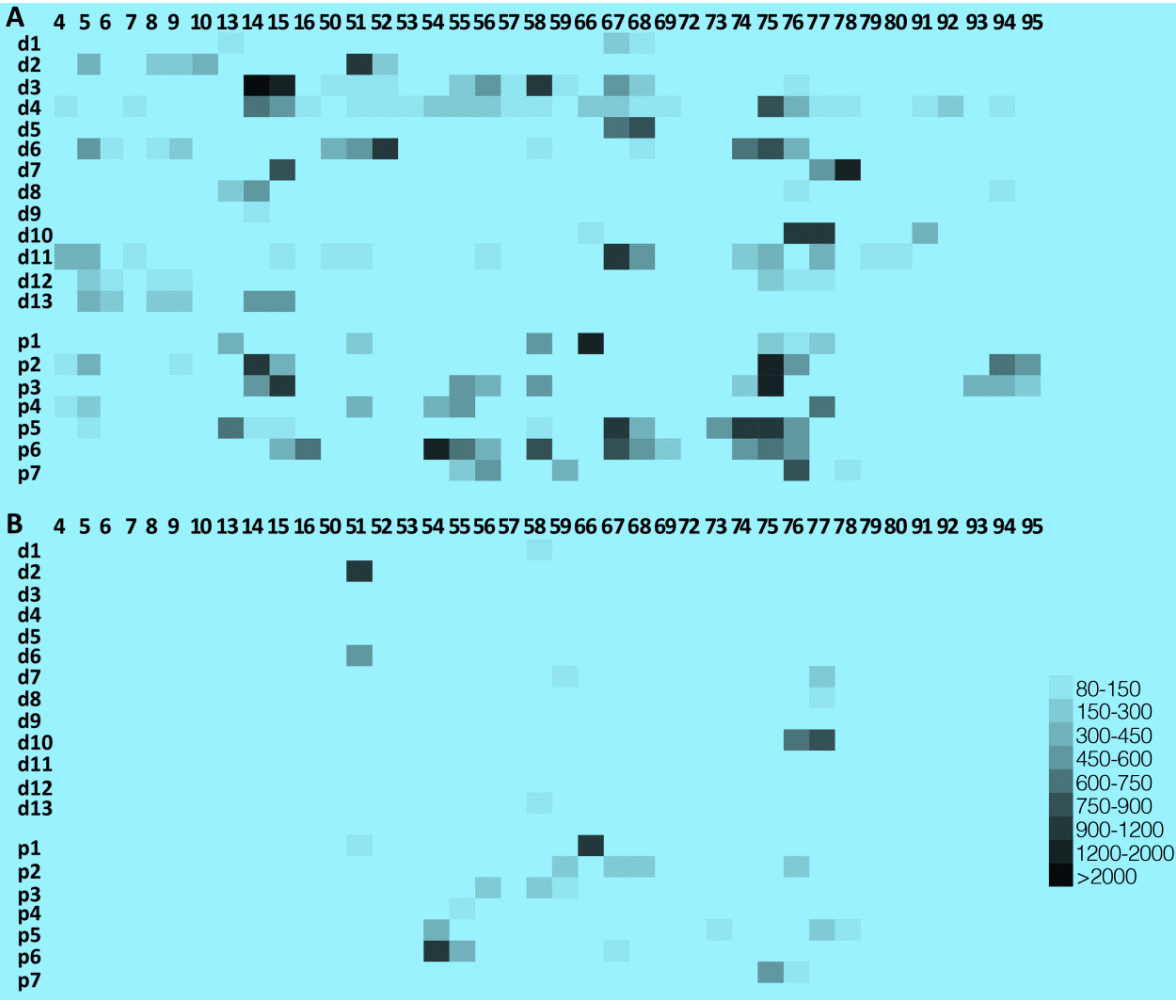
Mitigation Strategies for Immunogenicity

- **Engineer the patient's immune response**
  - Immune suppression
  - **Immune tolerance induction: antigen specific or antigen targeted**
  - Immune tolerance induction using globally immune suppressive regimens appropriate in some (eg autoimmune), but not all (eg cancer) clinical scenarios
- Engineer the therapeutic protein to be less immunogenic
  - Remove T/B cell epitopes in inherently immunogenic proteins
  - Develop products that have the same MOA but lack sequence/epitope homology to therapeutic counterpart of endogenous protein
  - Alter propensity to aggregate, deamidate, oxidize etc
  - Pegylation, Xtenylation or other means to shield epitopes and extend PK

Antigen Targeted Approach: Synthetic Vaccine Particles (SVP) Containing Rapamycin for Tolerance Induction

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- Material composition: PLGA
  - Biodegradable (lactic acid and glycolic acid)
  - used in multiple FDA-approved drugs
- Self assembly
- Encapsulation of Rapamycin (SVP-Rapamycin)
- Preferential uptake by APC due to size (nano), shape and surface charge
- Successfully prevented immune response to Factor VIII, and pegsiticase in mice, rats and monkeys (Zhang et al. Cell Immunol, 2016 , Kishimoto et al. Nat Nanotechnol, 2016)
- Safety profile documented in humans: Multi-Dose Safety/Pharmacodynamic Study of SEL 212/SEL-037 in Subjects With Symptomatic Gout &Elevated Blood Uric Acid (NCT02959918)

PLA / PLA-PEG

Rapamycin

SVP
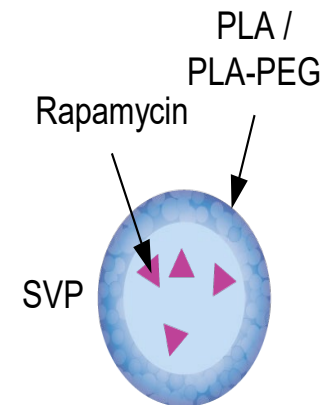
Recombinant Immunotoxin + SVP-RAPA Induces Tolerance and Prevents Formation of Neutralizing ADA in Mice

Mazor et al. PNAS 2018
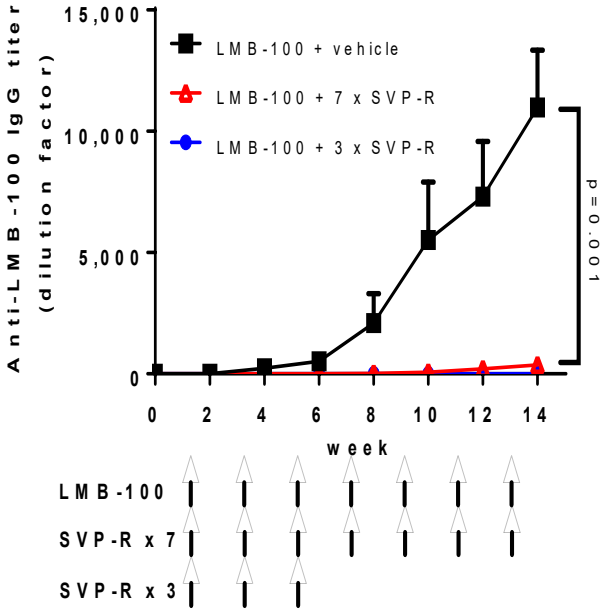
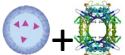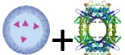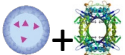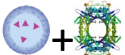# Severe Chronic Gout: A Debilitating Disease

- Severe gout is caused by accumulation of uric acid crystals in joints and soft tissue causing inflammation and pain

- Therapeutic goal in gout is to reduce serum uric acid levels below 6 mg/dL

Uricase

Uric acid begins to crystalize above 6.8 mg/dL

Serum Uric Acid (mg/dL)

6.0

Time
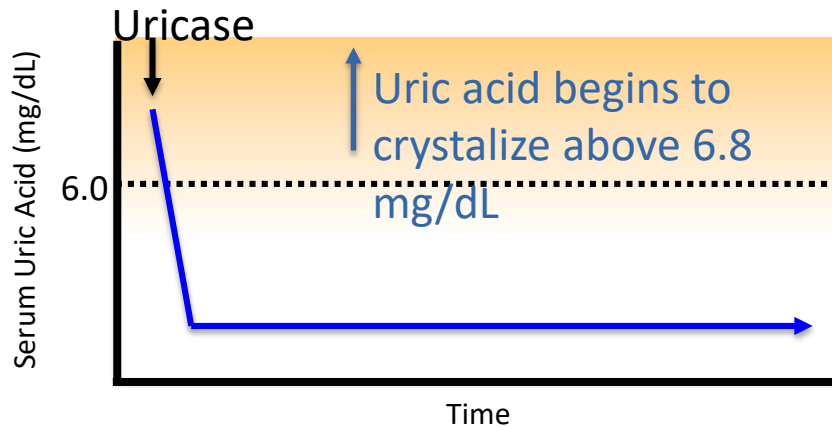
- All animals except for humans and greater apes have endogenous uricase

- Recombinant uricase is an effective therapy to eliminate uric acid, but is highly immunogenic in humans

SELECTA
Biosciences

Clinical Activity of ImmTOR (SVP-RAPA)+ Pegadricase

0.4 mg/ kg Pegadricase only

0.03, 0.1, 0.3 mg/kg ImmTOR only

0.03 mg/kg ImmTOR
0.4 mg/kg Pegadricase

0.10 mg/kg ImmTOR
0.4 mg/kg Pegadricase

0.15 mg/kg ImmTOR
0.4 mg/kg Pegadricase
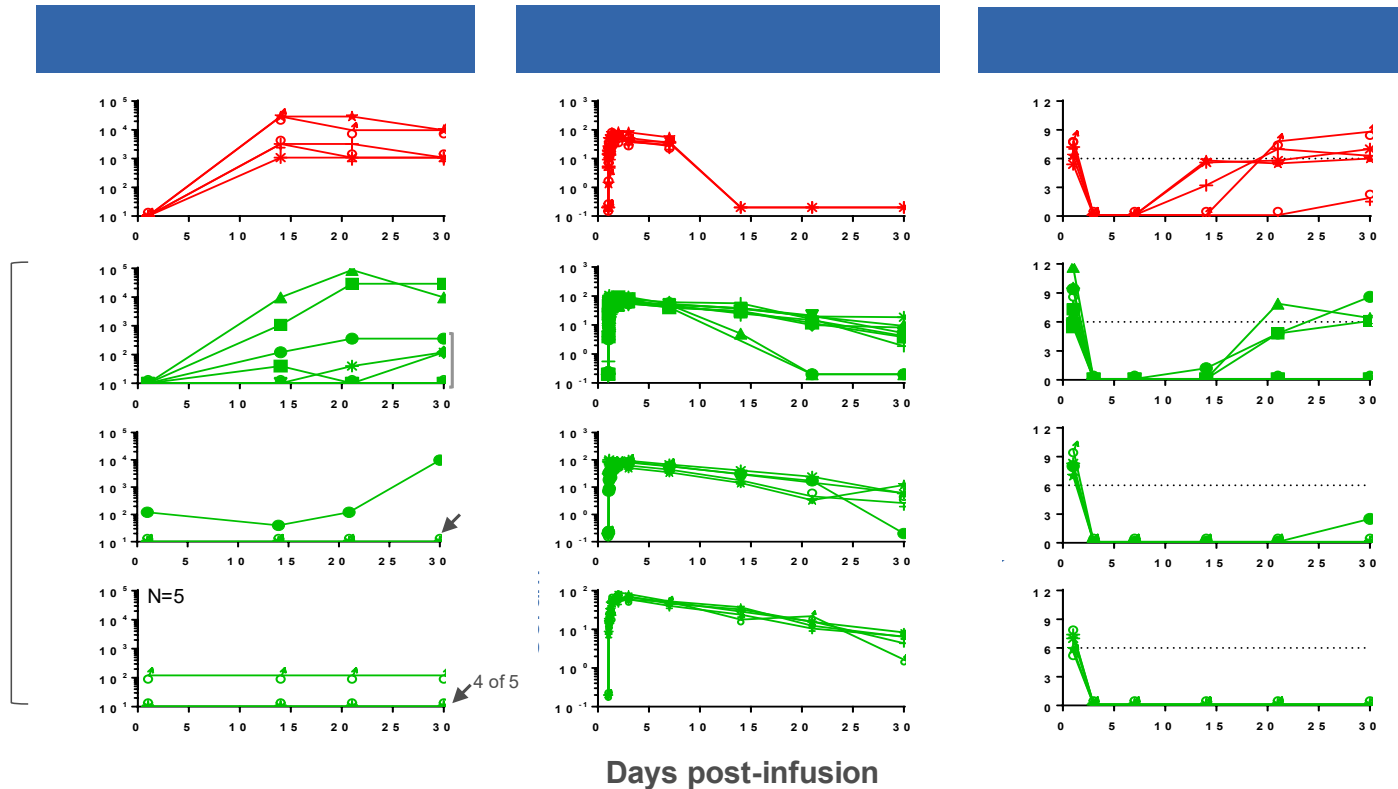
0.30 mg/kg ImmTOR
0.4 mg/kg Pegadricase
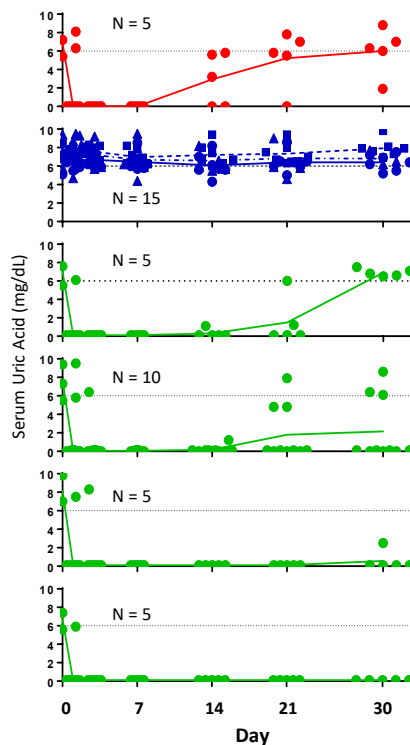
Loss of control over serum uric acid levels by day 14

No effect on serum uric acid levels

Dose-dependent reduction in serum uric acid levels

Correlation Between ADA Titers, Pegadricase Activity and Serum Uric Acid Levels

Days post-infusion

Clinicaltrials.gov NCT02648269

Inverse correlation between SAE IRs and the dose of SVP-RAPA

Acknowledgements

- Priya Kishnani, Zoheb Kazi, Ankit Desai, Duke University

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- Janet Woodcock, Director, CDER, FDA