



# **Risk Assessment and Mitigation Strategies for Immune Responses to Therapeutic Proteins: the FDA Perspective**

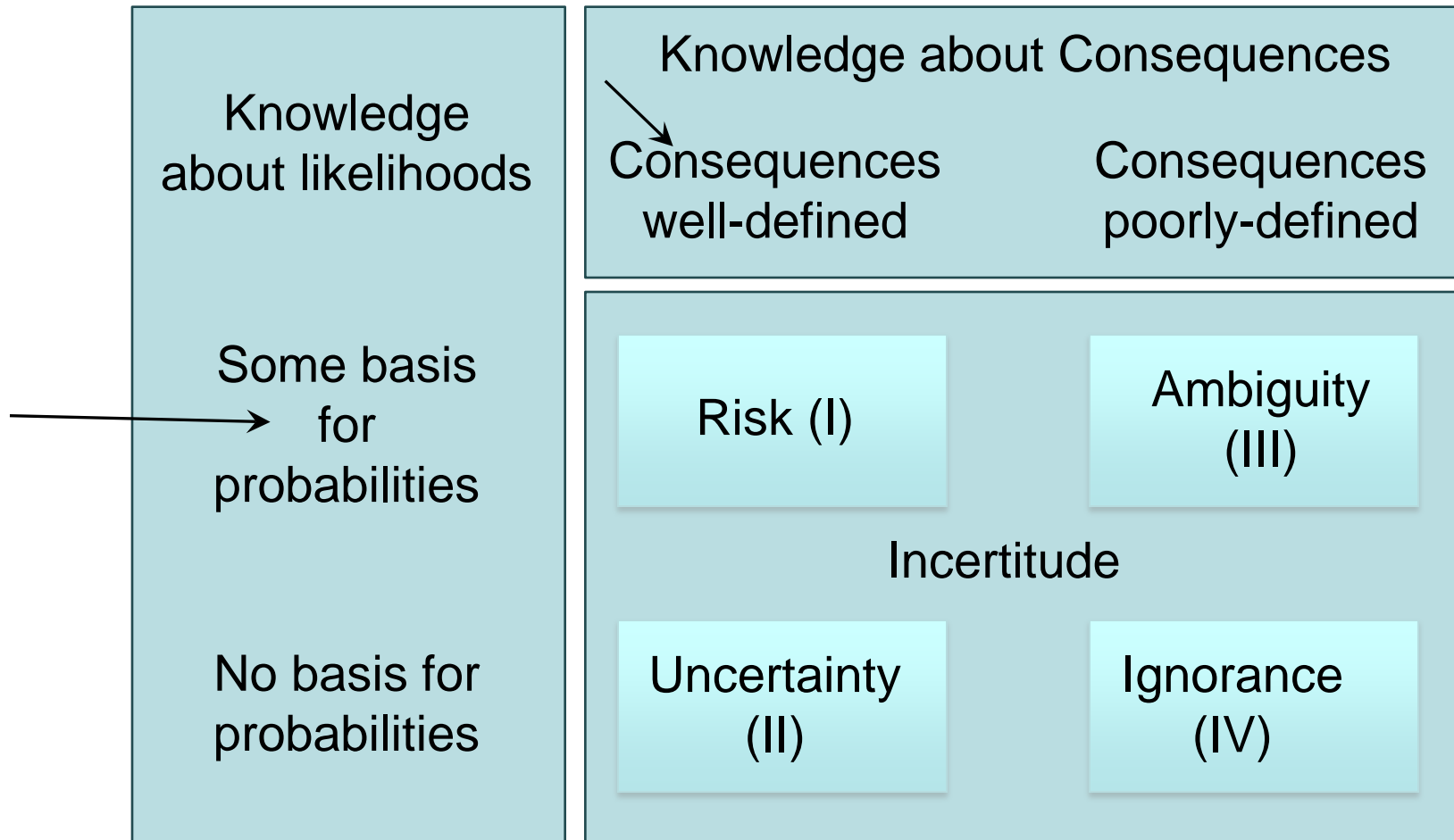
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Biotechnology Products

CDER, FDA

# Risk is a Specific Knowledge Set Encompassing Both Consequences and Probabilities

(modified from Stirling and Gee 2002)





# **“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 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
  - Cross reactive neutralization of endogenous factor or receptor homolog with unique function resulting in deficiency syndrome or cytokine release syndrome
  - Immune complex disease and delayed hypersensitivity
    - Serum sickness; nephropathy
    - Most often seen when high doses of therapeutic proteins are administered in setting of a sustained high titered 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;
  - Sustained or increased anti-drug antibody in the face of continued or escalated treatment dosage/frequency of product may lead to
    - epitope spread and generation of neutralizing antibodies
    - immune complex disease
- No apparent effect
  - But sustained response may lead to epitope spread and generation of neutralizing responses
    - IL-2
    - IFN- $\beta$

# Mitigation Strategies for Immunogenicity

- Engineer the patient's immune response
  - Immune suppression
  - Immune tolerance induction
- Engineer the therapeutic protein to be less immunogenic
  - Remove T/B cell epitopes
  - Develop products that have the same MOA but lack sequence/epitope homology to therapeutic counterpart of endogenous protein
  - Diminish PQAs associated with immunogenicity, eg propensity to aggregate, deamidate, oxidize etc
  - Pegylation, Xtenylation or other means to shield epitopes and extend PK

# Immunogenicity Risk Assessment: Consequences for Safety

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    - Cytokine release syndromes
  - Cross reactive neutralization of endogenous factor or receptor homolog with unique function resulting in deficiency syndrome or cytokine release syndrome
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# Mitigation of IgE Mediated Anaphylaxis to a Life Saving Therapeutic Enzyme by IgE mAb

(Rohrbach et al J. Inherit Metab Dis 2010)

- Patient with Pompe Disease for which a rh- $\alpha$ glucosidase enzyme replacement therapy (ERT) is *life saving*
- Patient had persistent, severe IgE mediated life-threatening allergic reactions to rh- $\alpha$ glucosidase that were not controlled with corticosteroids, antihistamines, and decreased infusion rate.
- The regimen was optimized by adding omalizumab, a recombinant monoclonal antibody against IgE, at the age of 6 months.
- One year later, ERT intervals could be extended to 14 mg/kg every 10 days ***and the anti-allergic treatment was successfully weaned with the exception of omalizumab.*** Recombinant human GAA IgG antibody titers were measured routinely every 4–5 months and were always <1:800.
- ***Continuation of omalizumab necessary? Did anti-IgE treatment preclude generation of high titer IgG response?***

## Prevention/Mitigation of Cytokine Release Syndrome

- Suspicion: Mabs specific for cell surface receptors or for cell membrane expressed cytokines, as well as antibodies that develop in patients to therapeutic protein products that bind to cell surface receptors, have the potential to augment a product's intrinsic agonist activity and exacerbate infusion-related toxicities.
  - In vitro assessments of cellular activation in human whole blood or peripheral blood mononuclear cells, including proliferation and cytokine release, can help in overcoming the known limitations of animals in modeling activating stimuli in some T-cell subsets
  - an initial starting dose below that obtained by traditional calculations, slower infusion rates, and staggered dosing (repeat administration) in individual patients and in dosing cohorts recommended
  - Pre- and post-administration levels of C-reactive protein and cytokines; TNF- $\alpha$ ; IL-2; IL-6; IL-10; and IFN- $\gamma$  should be collected for products with potential for CRS; clinical signs and symptoms, such as an acute elevation of body temperature, erythema, and hypotension, may serve as markers of a proinflammatory response pertaining to cytokine release
  - Consider use of anti-IL-6, anti-TNFs: experience with CAR T cells

## Immunogenicity Risk Assessment: Consequences for Safety

- Anti-drug antibody can have severe consequences if it **cross-reacts to and inhibits a non-redundant endogenous counterpart of the therapeutic protein or related proteins**
  - Pure Red Cell Aplasia (PRCA) induced by antibody response to recombinant erythropoietin: sole factor mediating RBC production
  - Thrombocytopenia induced by antibody response to recombinant thrombopoietin: sole factor mediating platelet production
  
- Theoretically, anti-drug antibody could cause activation induced effector function, cytokine release, or cell death when therapeutic protein product is a receptor whose endogenous counterpart is expressed on cells
  - IL-1R based products
  - TNFR based products

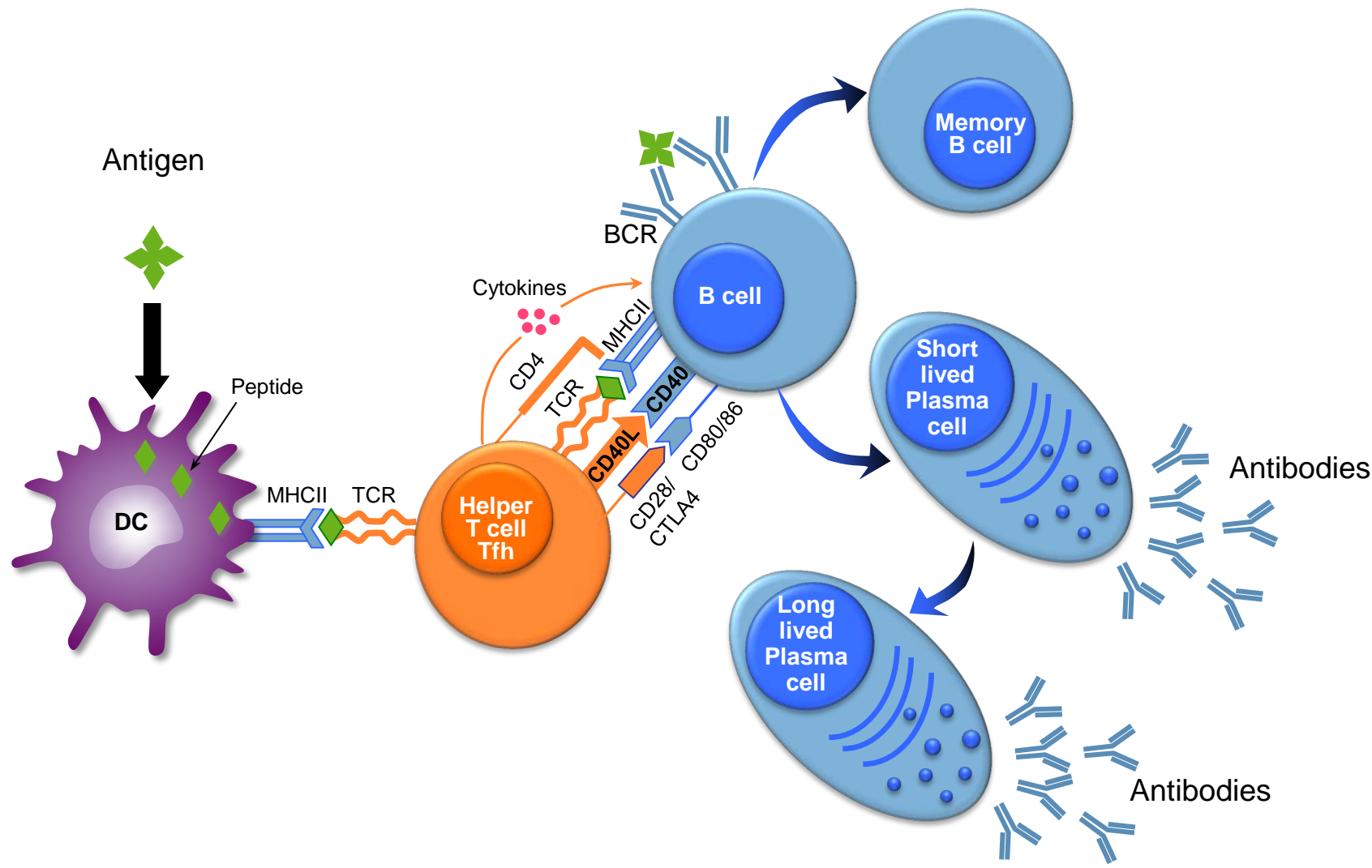
## How Human is Human, Self is Self?

Self proteins can be immunogenic and tolerance to them broken by administration of a therapeutic homolog. Immunogenicity of self proteins depends largely on:

- **Abundance:** determines the degree to which developing, potentially autoreactive T and B cells are tolerized
- **Alteration** in chemical/physical structure: aggregation, post-translational modifications (PTMs), chemical degradation
- **Adjuvants:**
  - **Extrinsic:** innate immune response modifiers
  - **Intrinsic:** immunomodulatory properties of the protein



# Antibody Response to Proteins: The Mediators



# T Cells More Robustly Tolerant to Self-Proteins: Thymic Mechanisms

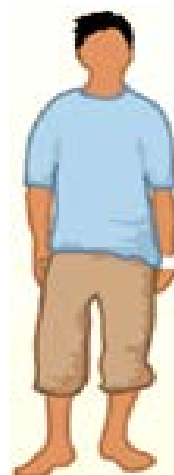
- **Natural (thymically generated) regulatory T cells (Tregs)**
  - Lineage deviation of T cells with high affinity to self proteins expressed in thymus to immune suppressive regulatory T cells (Tregs) distinguished by FOXP3 transcription factor, neuropilin and helios
  - Mutations in FOXP3 or in AIRE give rise to autoimmunity: immune dysregulation, polyendocrinopathy, enteropathy; X-linked (IPEX); autoimmune polyendocrine syndrome 1
- **Peripherally generated Tregs (Tp, Tr1, Tr3)**
- **Negative selection in thymus**
  - T cells with high affinity for thymic/peripheral tissue antigens that access thymus in sufficient quantities
  - T cells with high affinity for peripheral tissue antigens whose expression in thymus is mediated by transcription factor AIRE

# Mutations in Regulatory T cell Transcription Factor FoxP3 Confer Autoimmunity by Deficiency of Tregs

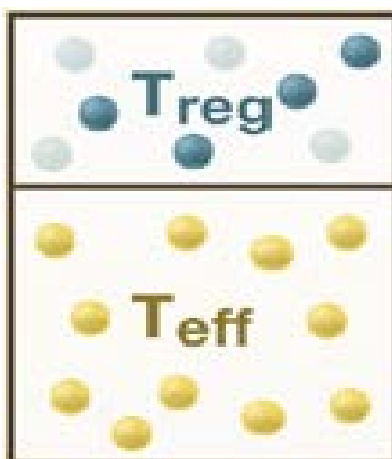
(Sakaguchi et al 2008)



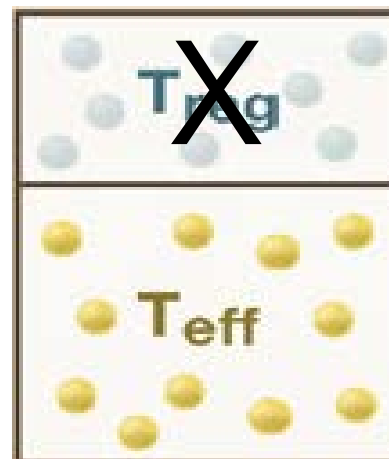
Mother of IPEX patient



IPEX patient



Normal



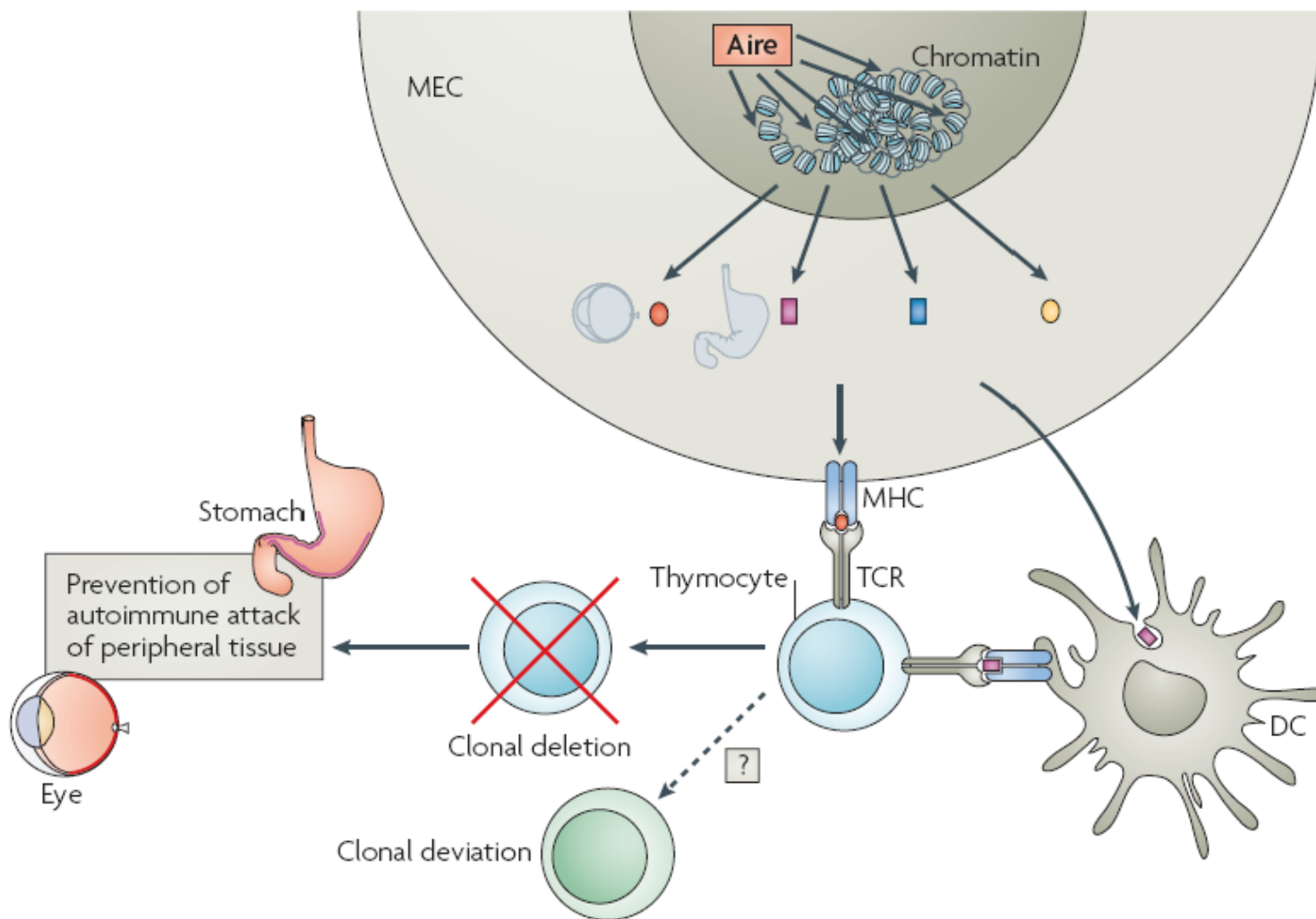
Autoimmune disease

Inflammatory bowel disease

Allergy

# Autoimmune Regulator (AIRE) Promotes Expression of Peripheral Tissue Antigens in the Thymus, Deleting/Deviating High Affinity Autoreactive T cells

(Mathis and Benoist 2007)



# AIRE Mutations are Associated with Autoimmune Polyendocrine Syndrome Type 1 (Kampe et al 2008)

**Table 1.** Associations between Clinical Manifestations of Autoimmune Polyendocrine Syndrome Type 1 (APS-1) and the Presence of NACHT Leucine-Rich-Repeat Protein 5 (NALP5) Autoantibodies.\*

Manifestation	APS-1	APS-1 and NALP5 Autoantibodies		P Value
		With Manifestation	Without Manifestation	
		<i>number/total number (percent)</i>		
Hypoparathyroidism	73/87 (84)	36/73 (49)	0/14	<0.001
Hypogonadism	28/87 (32)	19/28 (68)	17/59 (29)	<0.001
Adrenal insufficiency	69/87 (79)	29/69 (42)	7/18 (39)	0.81
Type 1 diabetes mellitus	11/87 (13)	2/11 (18)	34/76 (45)	0.10
Vitiligo	17/87 (20)	7/17 (41)	29/70 (41)	0.99
Alopecia	30/87 (34)	11/30 (37)	25/57 (44)	0.52
Hepatitis	15/87 (17)	8/15 (53)	28/72 (39)	0.31
Malabsorption	22/87 (25)	10/22 (45)	26/65 (40)	0.66
Pernicious anemia	14/87 (16)	5/14 (36)	31/73 (42)	0.64
Candidiasis	83/87 (95)	34/83 (41)	2/4 (50)	0.72

FOREIGN

SELF



- Low abundance self-protein
- **Aggregates** of self proteins
- **PTMs/chemical degradation** of self proteins
- **Adjuvants: external and internal**



**Expect  
Immunogenicity**

No tolerance  
Neutralize Product  
Hypersensitivity

**Potential  
Immunogenicity**

Incomplete tolerance  
Altered structure/  
Antigen Present  
Epitope spreading

**Rare  
Immunogenicity**

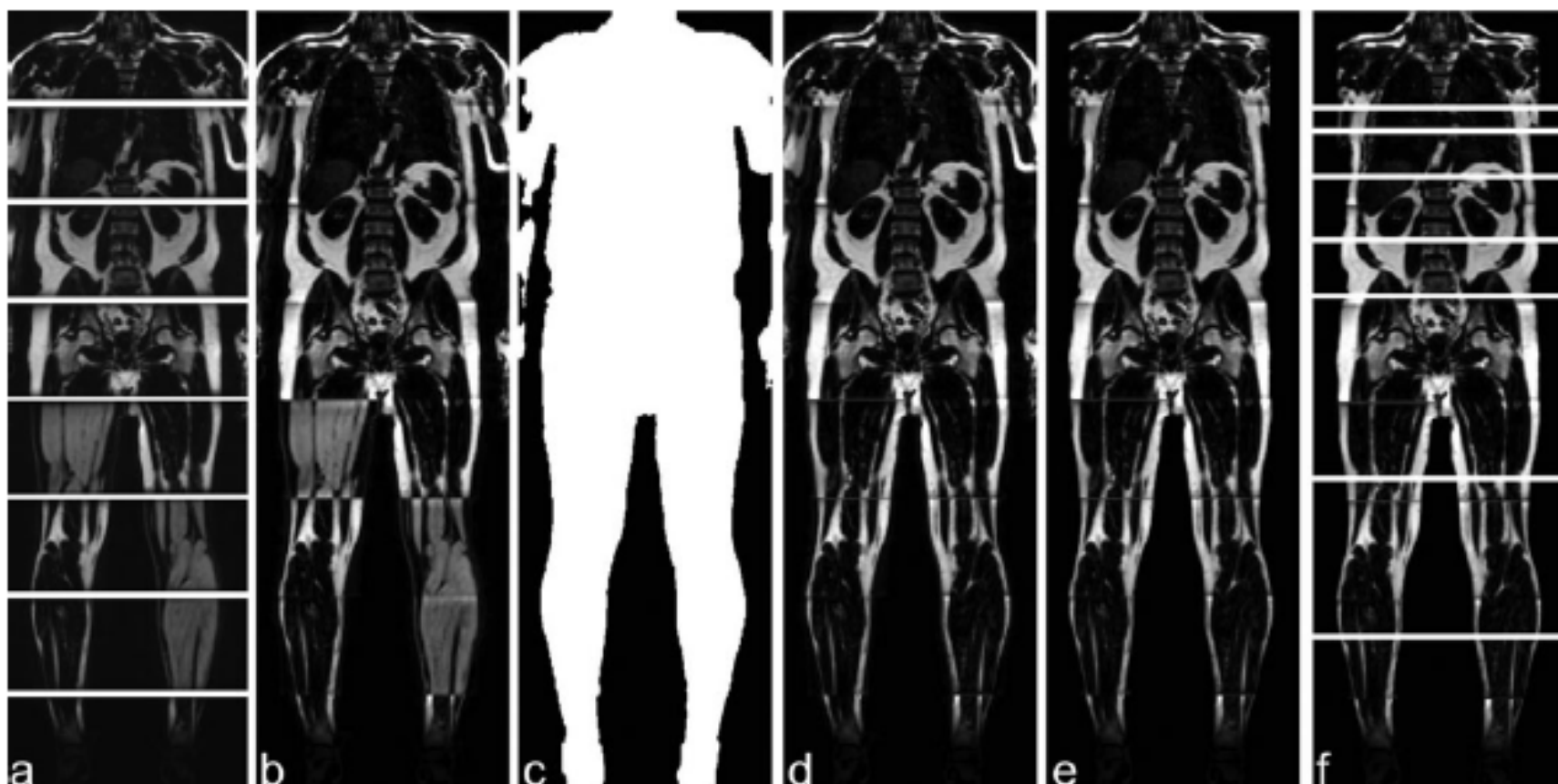
Robust tolerance  
Novel Route of Administration  
Adjuvants  
HLA Haplotype Specific

# Integrity of Protein Therapeutics in Storage Vessels Easily Monitored and Well Preserved



## Therapeutic proteins, well preserved ex-vivo, may be physically altered in in vivo environments: impact on immunogenicity

(from Wald D et al 2012)



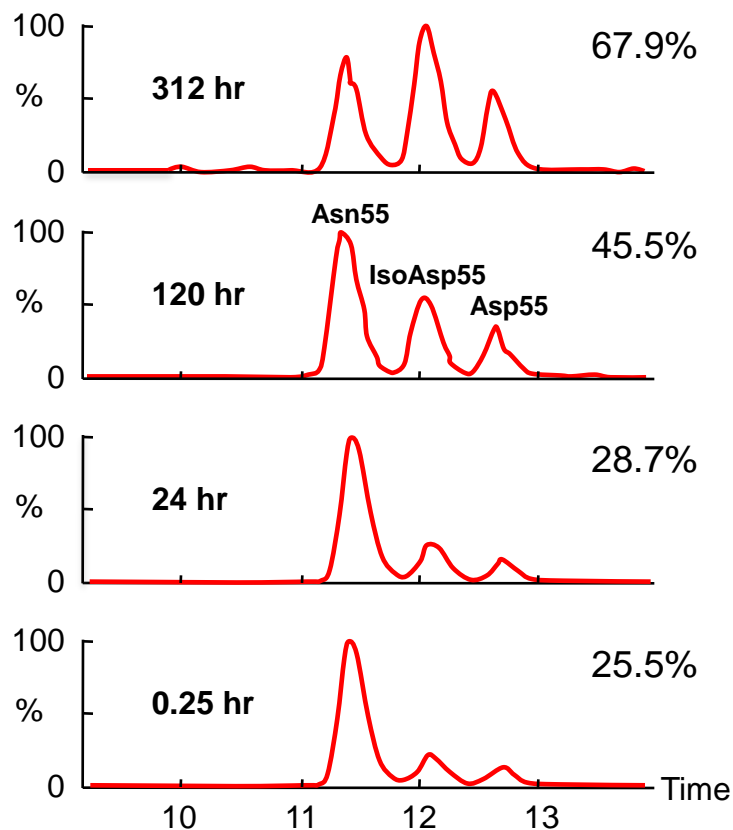
## Mechanisms by which the in Vivo Environment Influences Protein Immunogenicity

- Enhance product aggregation
- Alter antigen processing and presentation: alteration of sites and the extent/rapidity of protease activity
  - Generation of novel epitopes
- Enhance HLA binding of product related peptides: **deamidated and citrullinated** proteins generate peptides with enhanced class II HLA binding-strongly associated with autoimmunity

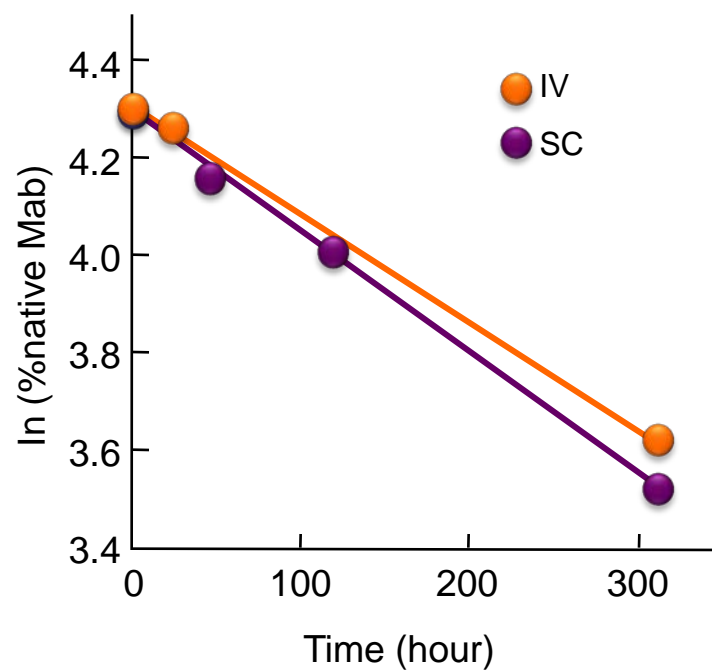
# Extensive and Rapid Deamidation of a MAb *in Vivo*: Effects on Activity and Immunogenicity?

(Huang L et al *Analyt. Chem* 2005)

Extracted Ion Chromatogram of Asn55  
Containing Peptide from MAb

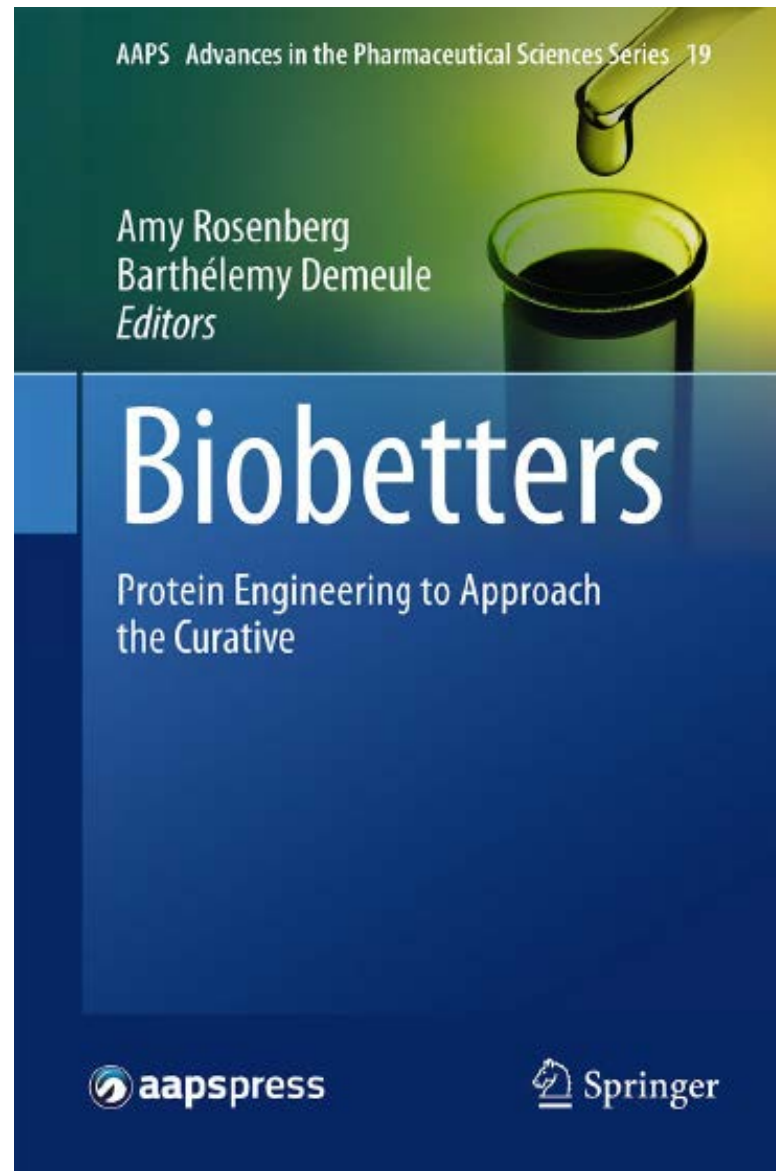


Rate of Asn55 Deamidation in Vivo



## **Assessment of the Fate of Therapeutic Proteins in Vivo: Vital for Biobetter Therapeutics**

- Biobetters: engineered therapeutic proteins for better efficacy and safety
  - Resist chemical degradation: loss of activity, increased immunogenicity
  - Better tissue targeting: eg muscle uptake of therapeutic enzymes, penetration of the CNS
  - Diminish immunogenicity



**FOREIGN**

**SELF**



- **Low abundance self-protein**
- Aggregates of self proteins
- PTMs or chemical degradation of self proteins
- Adjuvants



**Expect Immunogenicity**  
No tolerance  
Neutralize Product  
Hypersensitivity

**Potential Immunogenicity**  
Incomplete tolerance  
Altered structure/  
Antigen Present  
Epitope spreading

**Rare Immunogenicity**  
Moderate to High Abundance  
Novel Route of Administration  
Adjuvants  
HLA Haplotype Specific

## Low Abundance Self Proteins: Break in Tolerance to Thrombopoietin

- Levels of TPO in healthy volunteers in  $10^{-12}$  M range:
- N-terminus contains receptor binding domain; C-terminus contains three MHC class II binding motifs and one promiscuous immunodominant T cell epitope (Epivax analysis).
- Administration of **human** full length TPO to non-immune suppressed animals induced NABS and thrombocytopenia: ***attributed solely to contribution of xenogeneic determinants***
- Antibodies to full length TPO developed in 7% of treated *immune suppressed* cancer patients; few developed NAB, none developed thrombocytopenia.
- Administration of **species specific** full length TPO to non-immune suppressed monkeys and mice induced NABS and thrombocytopenia (G. Koren, Devel Biol 2002)
- Antibody response in animals developed initially to C-terminal and by epitope spread, to the N-terminal receptor binding domain.

## Mitigation Strategy 1 for TPO Immunogenicity: Development of C-Terminal Truncated and Pegylated TPO

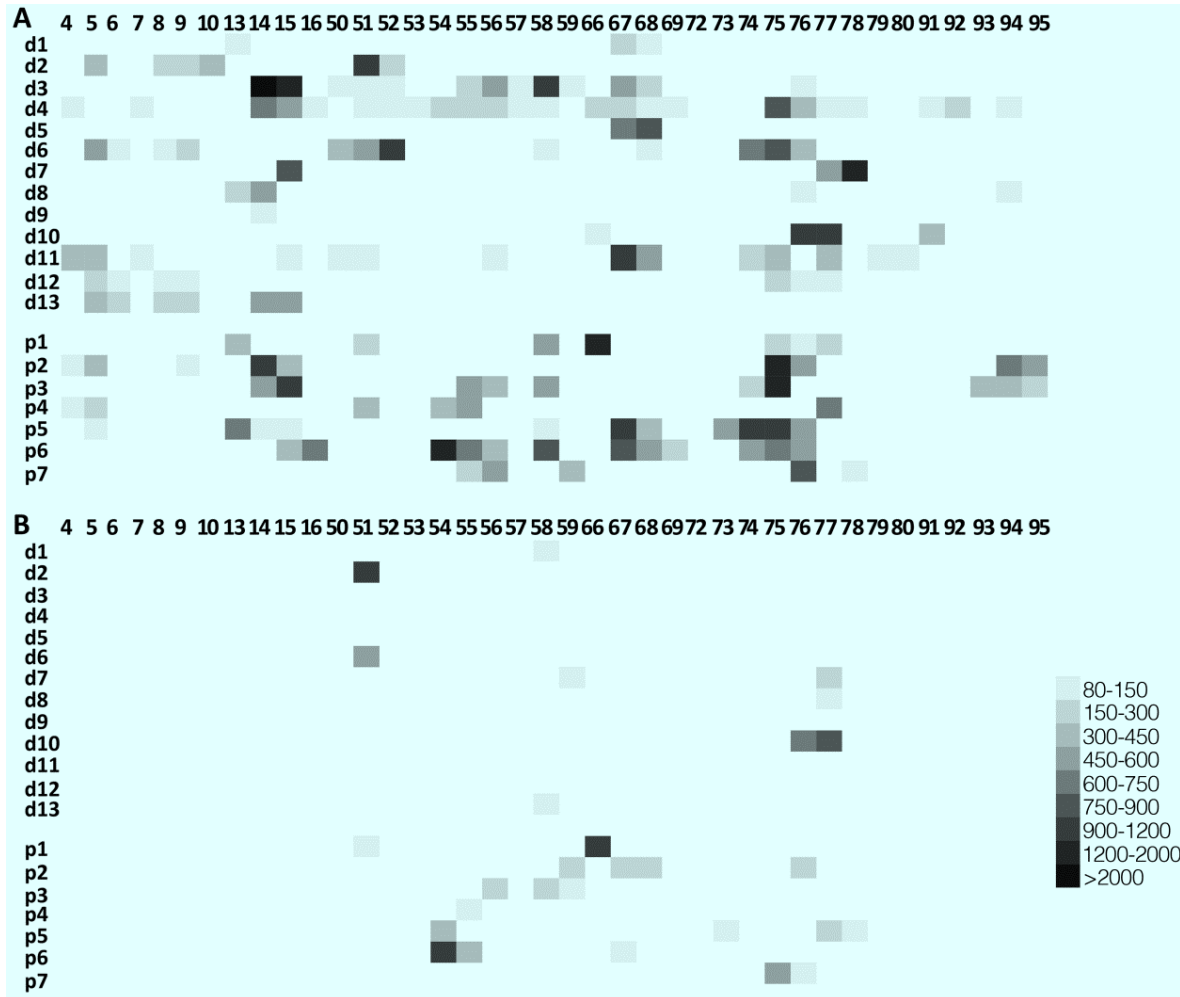
- Strategy for Mitigating Immunogenicity of TPO
  - Elimination of highly immunogenic C-terminus
  - Pegylation used to reduce immunogenicity and increase t<sub>1/2</sub> of therapeutic proteins: “shielding” epitopes or altering antigen processing and presentation
- Neutralizing antibody to PEG-MGDF caused thrombocytopenia in healthy platelet donors: 13/325 (4%) and in immune suppressed oncology patients (0.5%) (Li et al 2001)
  - In some healthy donors, tolerance was easily broken (2-3 doses); argues against epitope spread, even from initial PEG or N-terminal domain site, as mechanism
  - Common HLA alleles in two formerly healthy patients: HLA-DQB5 0302/7, HLA-DR B1 04 and HLA-DR B4 01. Analysis to identify both high binding epitopes and corresponding class II alleles might further elucidate immune pathogenesis.

## Mitigation Strategy 2 for TPO Immunogenicity: Development of Romiplostim

- Romiplostim, a member of the TPO *mimetic* class, is an Fc-peptide fusion protein (peptibody) that activates intracellular transcriptional pathways leading to increased platelet production via the TPO receptor (also known as cMpl).
- The peptibody molecule contains two identical single-chain subunits, each consisting of human immunoglobulin IgG1 Fc domain, covalently linked at the C-terminus to a peptide containing two thrombopoietin receptor-binding domains. **Romiplostim has no amino acid sequence homology to endogenous TPO.**

# Engineered Recombinant Immunotoxin has Dramatically Reduced T Cell Responses

(Mazor et al PNAS 2014)



## PRCA in Development of Biosimilar Epo: Suspect Lineup in the Search for the Smoking Gun

- Three cases of NABs and one case of PRCA in development of a biosimilar Epo: ***two implicated batches*** thoroughly analyzed for suspect PQAs
  - aggregates
    - Micelles of erythropoietin: polysorbate generated micelles
    - ***Tungsten leachates*** from tungsten used in needle hub formation: protein aggregation from tungsten oxides; shown previously to aggregate other therapeutic proteins
  - adjuvant material leaching from rubber stopper:
    - ***vultac*** responsible for cross linking of rubber protein; can cross link other proteins eg epo? no
    - vultac has adjuvant properties (Sharma et al 2004)



## Tungsten Mediated Aggregates Likely Root Cause of Induction of Epo Antibodies in Biosimilar Epo (Seidl A et al 2012)

Potential risk factor PQA	Suspect batches had higher levels than?			Literature available to suggest a role in immunogenicity	Involvement in current root-cause
	Other batches of study drug	Other batches not used as study drug	Reference product (Eprex®)		
<b>Drug product</b>					
<b>Aggregation</b>	<b>Yes</b>	<b>Yes</b>	<b>No</b>	Yes	<b>Most likely</b>
<b>Unfolded variants/ irreversible Dimers</b>	<b>Yes</b>	<b>Yes</b>	<b>Yes</b>	Yes	<b>Most likely</b>
Subvisible particles	No	No	No	Yes	Unlikely
<b>Inorganic leachates</b>	<b>Yes (tungsten)</b>	<b>Yes (tungsten)</b>	<b>Yes (tungsten)</b>	Yes*	<b>Most likely</b>
<b>Organic leachates</b>	<b>No</b>	<b>No</b>	<b>No</b>	Yes	<b>Highly unlikely</b>
Oxidation Met-54	No	No	No	No	Highly unlikely
Oxidation Trp-64	No	No	No	No	Highly unlikely
Deamidation/aspartate	No	No	No	Yes	Highly unlikely
Isomerization					
Degradation	No	No	No	Yes	Highly unlikely
Silicone oil	No	No	No	Unclear	Highly unlikely
<b>Polysorbate 80 micelles</b>	<b>No</b>	<b>No</b>	<b>No</b>	<b>No</b>	<b>Highly unlikely</b>

\* Known role in protein aggregation



# Increased Tungsten (W) Content Unique to Implicated Product Batches

(Seidl A et al 2012)

Batch	770307	780307	260108	270108	400208	770508	770508	140808	700106	8ESTE00	770508	750906	380409	060609
Sample type	RSM	RSM	CSS	CSS	CSC	RSM	CC6	Ref. sample	Placebo	Erypo® Ref.	RSM	Ref. sample	Ref. sample	Ref. sample
<b>Ω</b>	<LOD	<LOD	0.99	0.97	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.52
<b>Al</b>	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
<b>Si</b>	2.32	1.57	6.42	3.20	3.70	1.86	3.26	3.17	17.20	29.50	1.86	2.14	2.03	1.55
<b>Ca</b>	<LOD	0.52	0.78	<LOD	1.31	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
<b>Se</b>	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.23	<LOD	<LOD	<LOD	<LOD	<LOD

## Criticality of Traceability

(EMA Guideline on Immunogenicity Assessment of Biotechnology Derived Therapeutic Proteins 2015)

- “...traceability is especially important for adverse events related to immunogenicity. Traceability is important for both routine pharmacovigilance (collection of spontaneously reported adverse events) and additional pharmacovigilance activities. Appropriate measures to improve traceability, collection of brand name and batch number, should be taken.”

## Mitigation Strategy for Epo related PRCA: Alter the Product

- Alter the Product:
  - Eliminate Tungsten from prefilled syringes
    - Enhanced washing procedures
    - Change to alternative metal for syringe manufacture
  - Ensure Cold Chain Integrity
    - PRCA cases in Thailand revealed improper storage conditions by unauthorized vendors, associated with high levels of aggregates in the product
- For Patients with Syndrome:
  - Rigorous immune suppression restores self tolerance

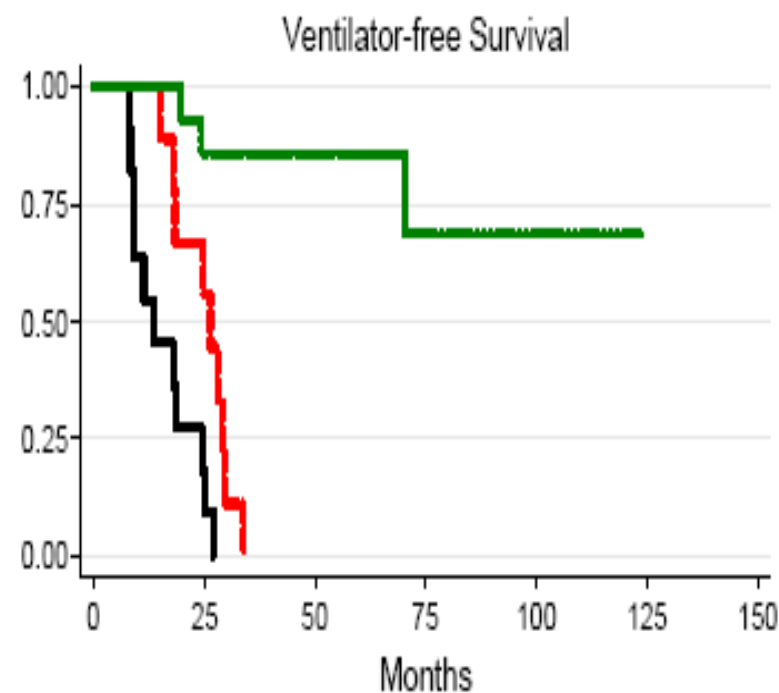
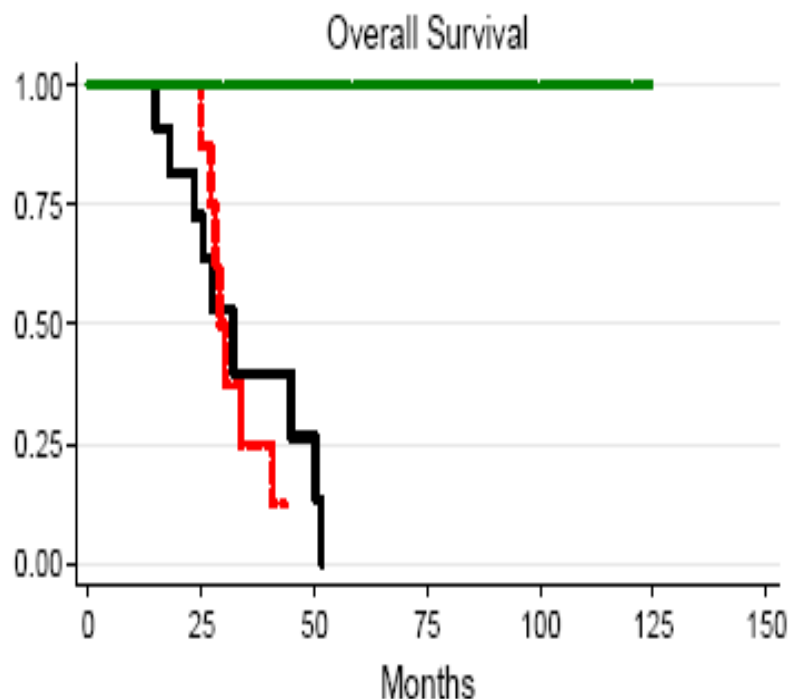
## Risk Mitigation: Guidance for Therapeutic Counterparts of Endogenous Proteins

- When developing therapeutic counterparts of self-proteins, an assessment of the uniqueness and extent of self-tolerance to the endogenous protein is recommended
  - Autoimmune syndromes in which self tolerance to factor is broken: eg PRCA associated with autoimmune disease
  - Effects of KO of protein in animal models to determine uniqueness; other animal studies (eg hyperimmunized animals)
  - Animal studies using species specific protein, ideally with similar formulation, process/product related impurities (Koren);
  - Acquire knowledge/assess abundance and secretion pattern (constitutive secretion/elicited) of factor; thymic expression (AIRE)
  - Pre-treatment evidence of immune response to endogenous protein: antibodies, T cell reactivity
  - Predictive algorithms/alanine scanning may identify immunogenic “hot spots”: potential for protein engineering
- Perform specific and sensitive antibody assays, including neutralizing antibody assays, in real time to assess safety of additional dosing (Assay Guidance)
- Carefully track lot disposition on a patient basis; implicated lots should be thoroughly evaluated for provocative factors

# 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
    - Metastable state? Sustained or increased response may lead to epitope spread and generation of neutralizing antibodies
    - Changes in dosing level and schedule may be required to maintain efficacy: caveats
- **No apparent effect**
  - But sustained response may lead to epitope spread and generation of neutralizing responses
    - IL-2
    - IFN- $\beta$

# Pompe Disease: High Titer Antibody Response Confers Negative Clinical Outcome in Enzyme Replacement Therapy-Treated Patients Disease

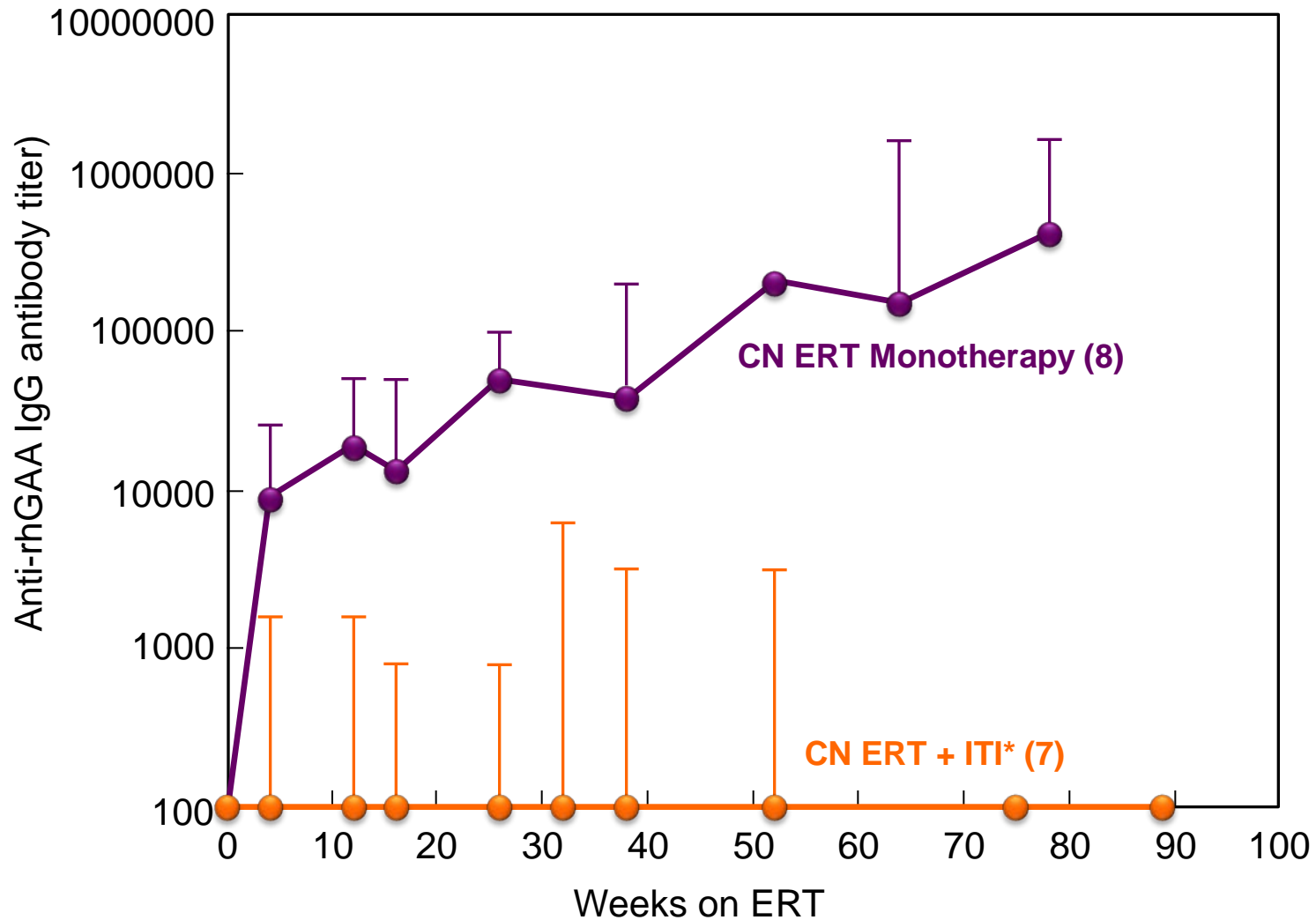


- High Titer CRIM+
- Low Titer CRIM+
- High Titer CRIM-

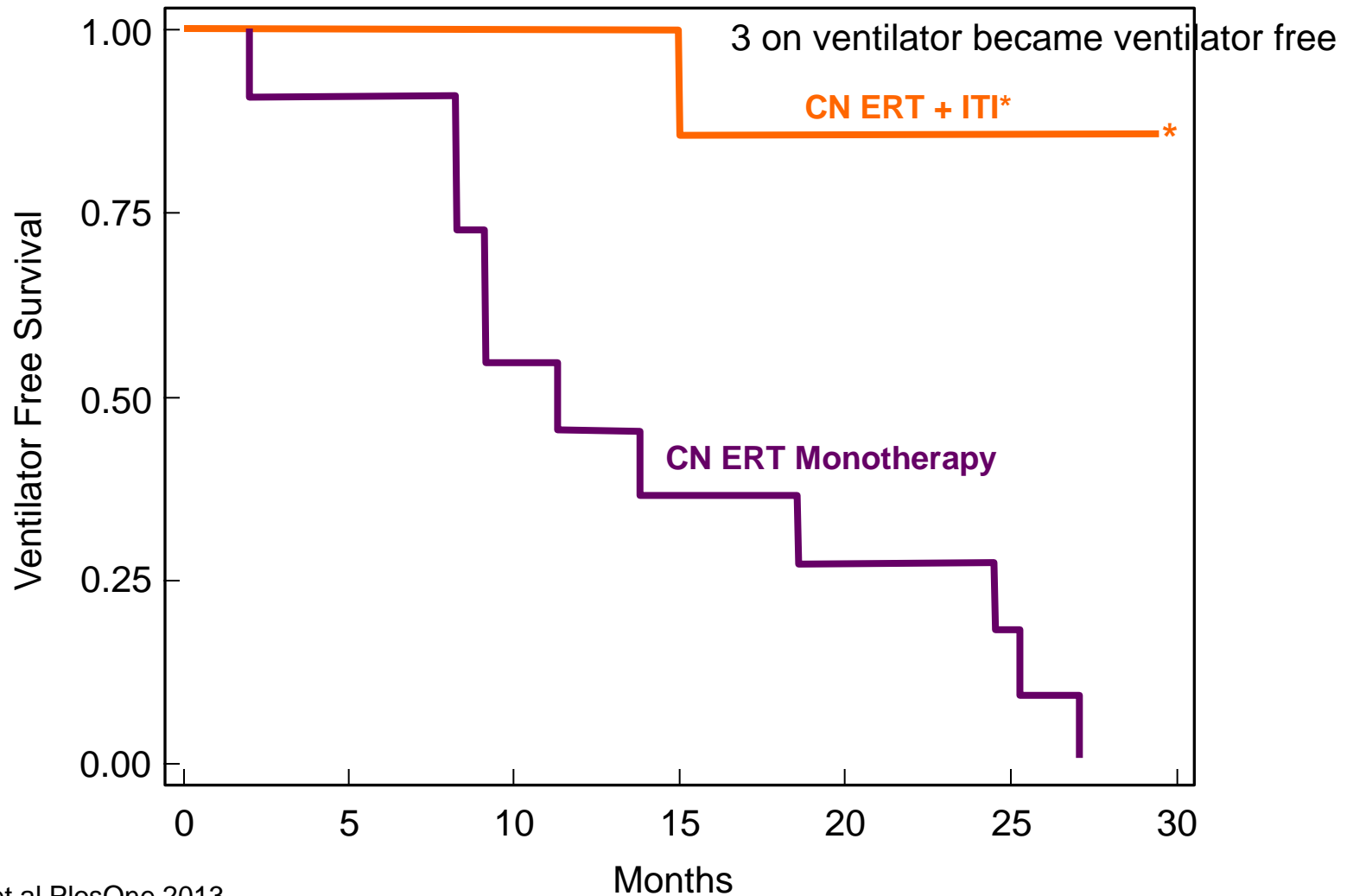
## Mitigation of Immune Response to Life Saving Therapeutics

- **When consequences are life threatening, tolerance induction may be lifesaving** (Kishnani PS et al Mol Genet and Metabolism 2016)
  - 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
- Protein engineering to “deimmunize” a protein therapeutic and development of mimetics that lack amino acid or epitope homology
  - 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 enhanced aggregation, oxidation, deamidation etc

## Efficacy of Prophylactic Immune Tolerance Induction on Response to ERT



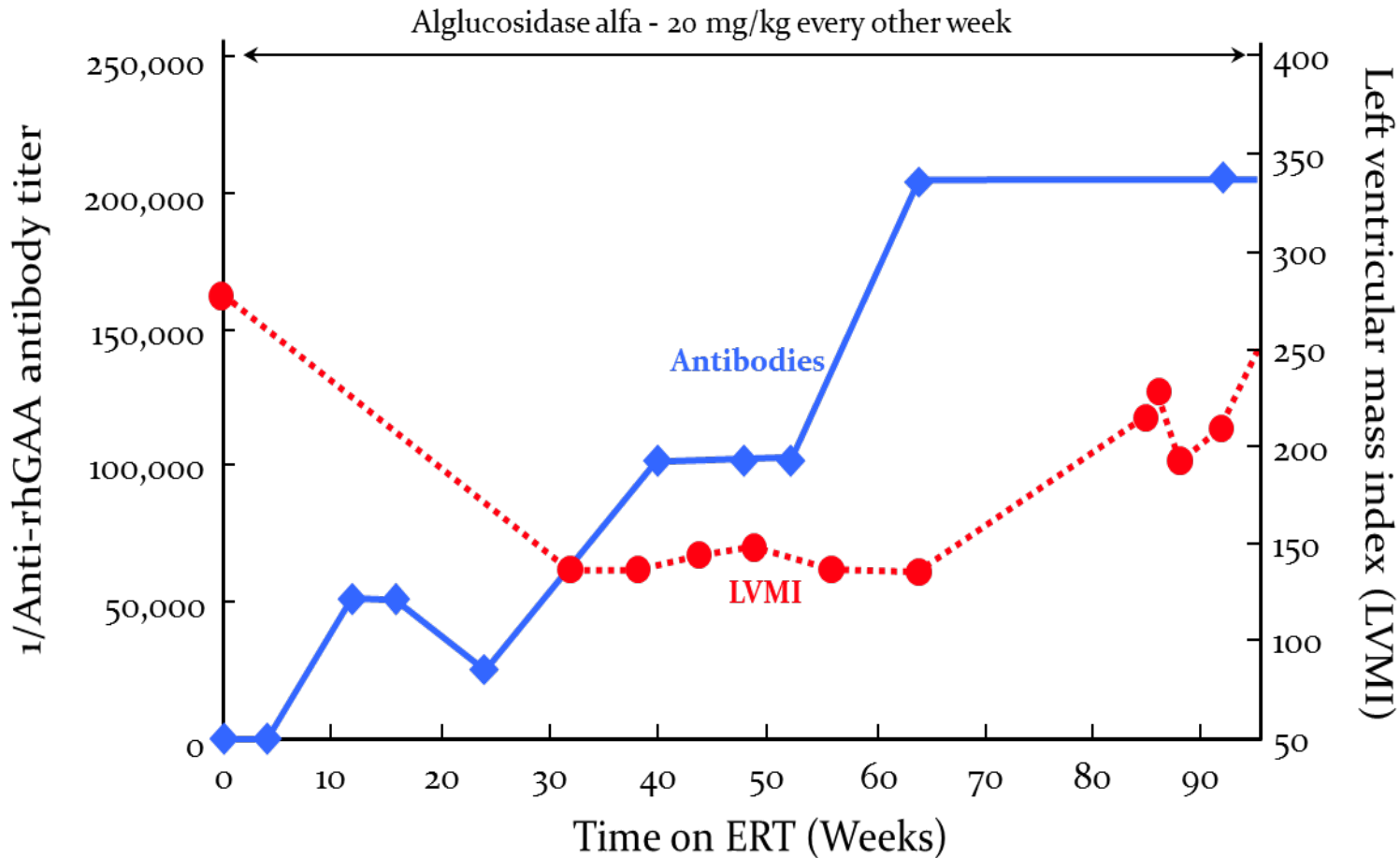
# Improved Ventilator Free Survival with ITI + ERT





# Entrenched Antibody Responses: Unresponsive to Immune Suppressive Agents

(PS Kishnani et al 2012)



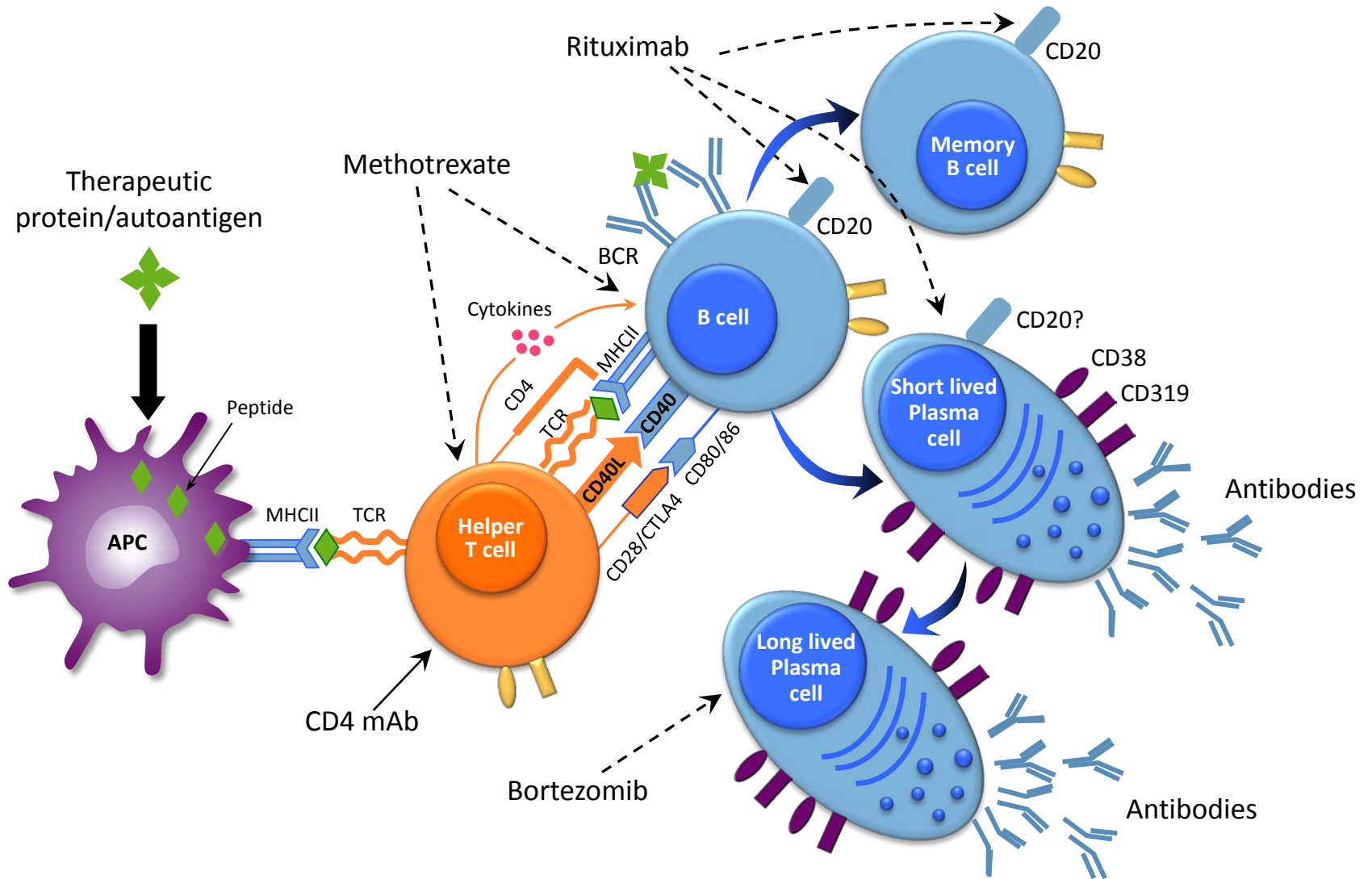
Cyclophosphamide (250 mg/m<sup>2</sup> IV)

Rituximab (375 mg/m<sup>2</sup> IV)

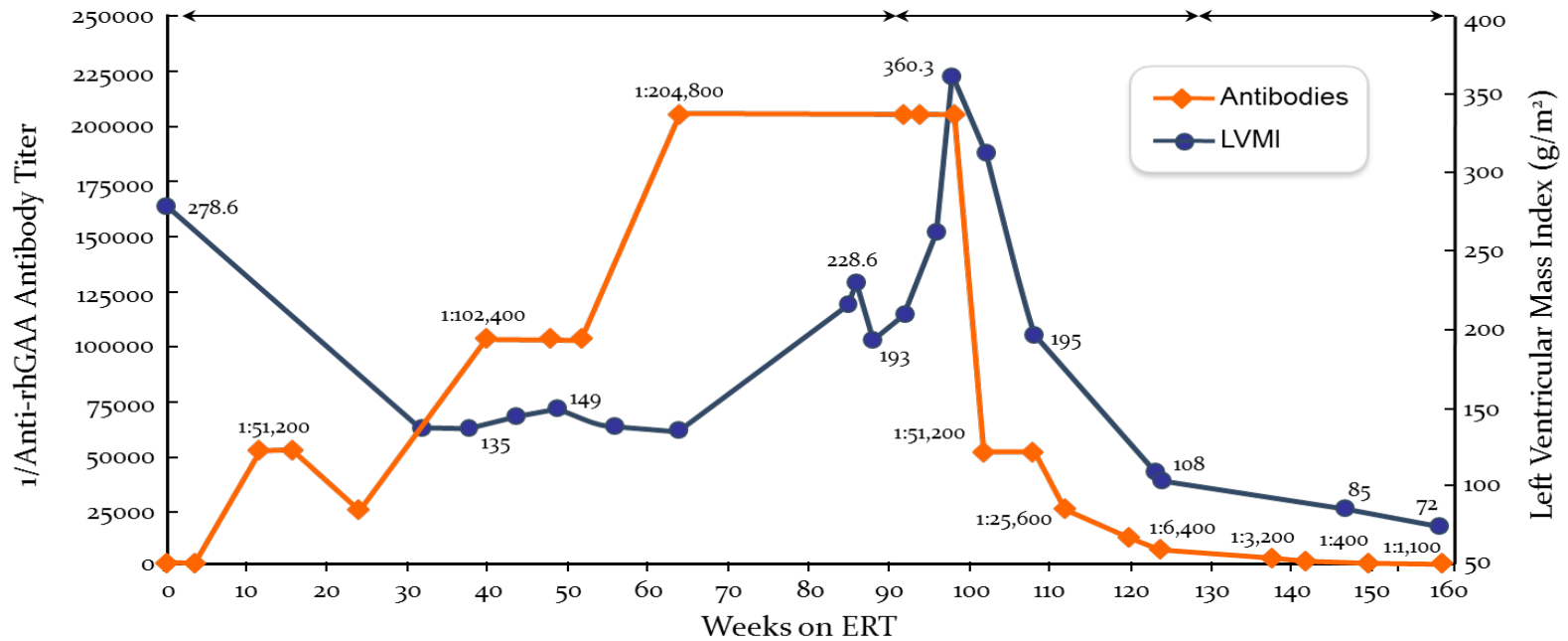
Methotrexate (15 mg/m<sup>2</sup> PO every other week)

IVIg (400-500 mg/kg IV monthly)

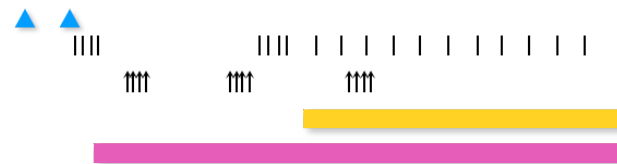




## Targeting Long Lived Plasma Cells with Bortezomib Reduces Antibody Titer in Patients with HSAT (Kishnani PS et al 2012)



Cyclophosphamide (250 mg/m<sup>2</sup> IV)  
Rituximab (375 mg/m<sup>2</sup> IV)  
**Bortezomib (1.3 mg/m<sup>2</sup> IV)**  
Methotrexate (15 mg/m<sup>2</sup> SC)  
IVIg (400-500 mg/kg IV monthly)



## Autoimmune Disease

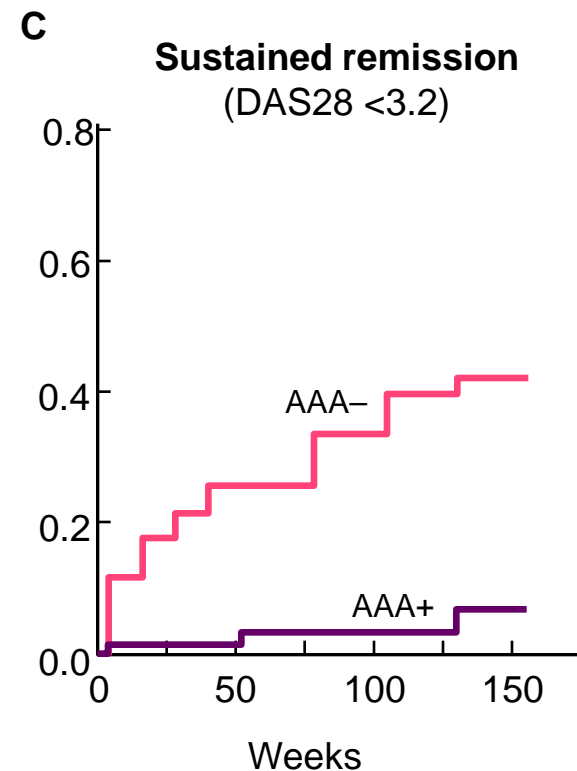
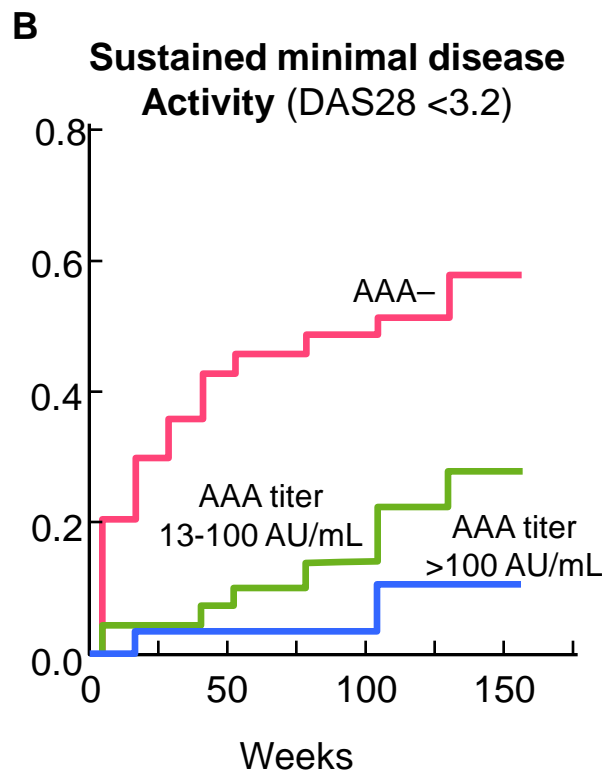
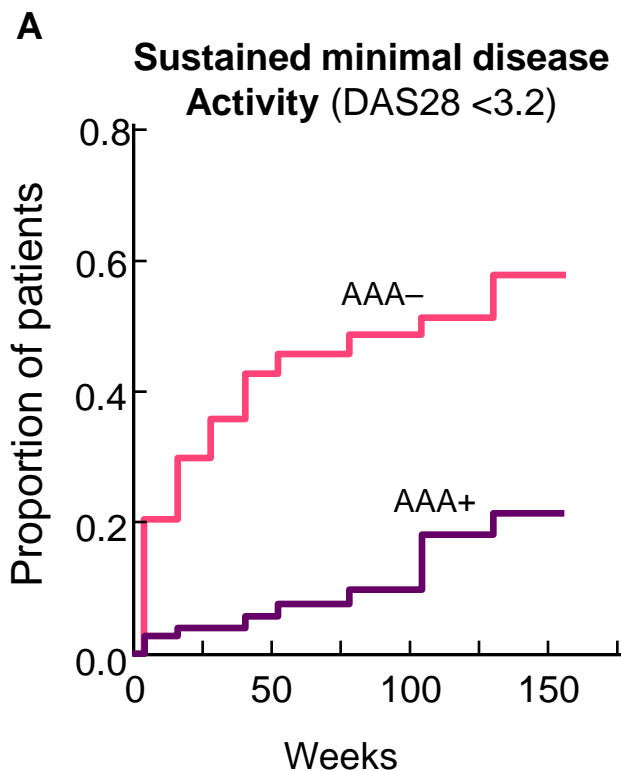
### Mediated by Antibodies: Can Targeting Long Lived Plasma Cells Improve Clinical Outcome?

(Rosenberg AS et al Clin. Immunol. 2016)

- Acquired Pulmonary Alveolar Proteinosis: autoantibodies to GM-CSF
- Anti-Synthetase Syndrome: autoantibodies generated against enzymes that acetylate transfer RNA
- Thrombotic Thrombocytopenic Purpura: autoantibodies against ADAMTS13
- Myasthenia Gravis: autoantibodies against neuromuscular junction proteins
- Systemic Lupus Erythematosus: autoantibodies directed against proteins and nucleic acids

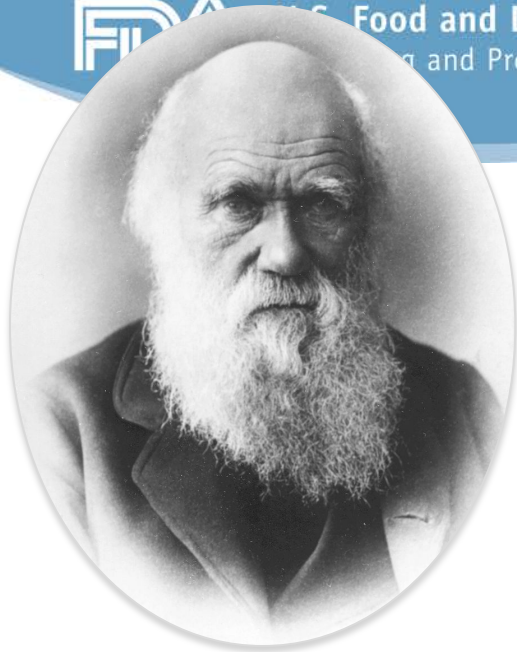
# Antibodies to Adalimumab Diminish Remission in RA: Consider Tolerance Induction at Outset of Treatment?

(Bartelds G et al JAMA 2011)



## Diminished Immunogenicity/Enhanced Efficacy of Concomitant Immunosuppressive Treatment in Autoimmune Disease: Is there a Downside?

- No difference in rate of serious infections: eg 4-5% in all groups (Colombel et al 2010). ***Requirement for steroid pulses heightens infectious risk.***
- Combination of azathioprine and anti-TNF biologic agents increases the relative risk of hepatosplenic T-cell lymphoma. Identifiable subset of patients at higher risk.
- Are patients who receive concomitant immunosuppression, especially MTX, immune tolerant to TNF mAbs? Treg population specific for mAbs?
- Would shorter, more intense 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?



## What Would Darwin Ask? How Foreign is Foreign?

- Many foreign (eg animal and microbial) sequence derived therapeutic proteins contain significant sequence homology to human proteins: does presence of foreign elements pose risk for breaking tolerance to conserved human sequences/domains?
  - Response to foreign domains could break tolerance to conserved, human domains via provision of T cell help to autoreactive, reversibly anergic B cells and/or by epitope spread.
  - Reciprocally, tolerance to conserved domains could theoretically induce tolerance to foreign domains via linked suppression/dominant tolerance, mediated by T regulatory cells.

## FOREIGN

**Foreign epitopes**



**Expect Immunogenicity**  
Immune response to foreign  
determinants affects tolerance  
to self-determinants?

## SELF

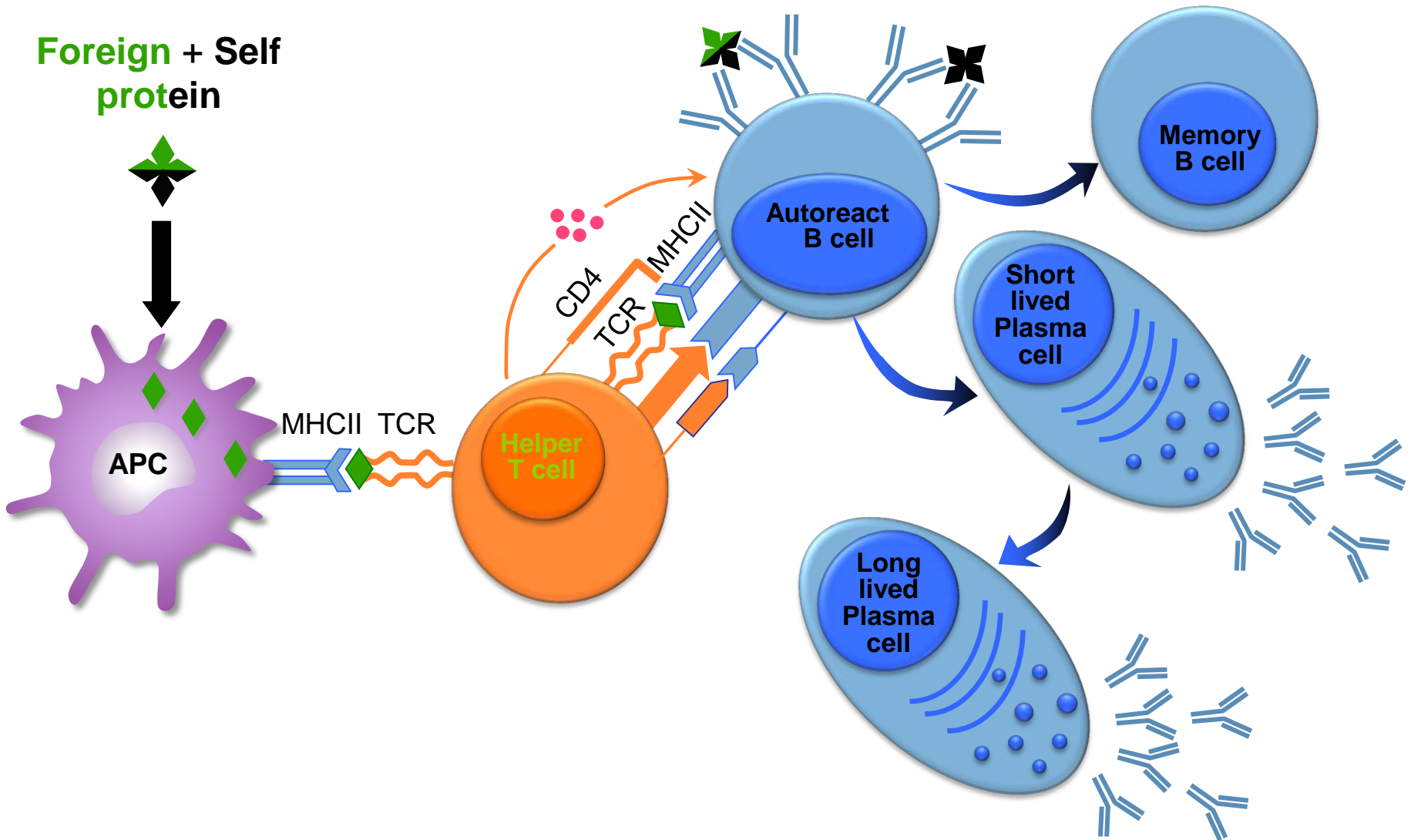
**Self epitopes**



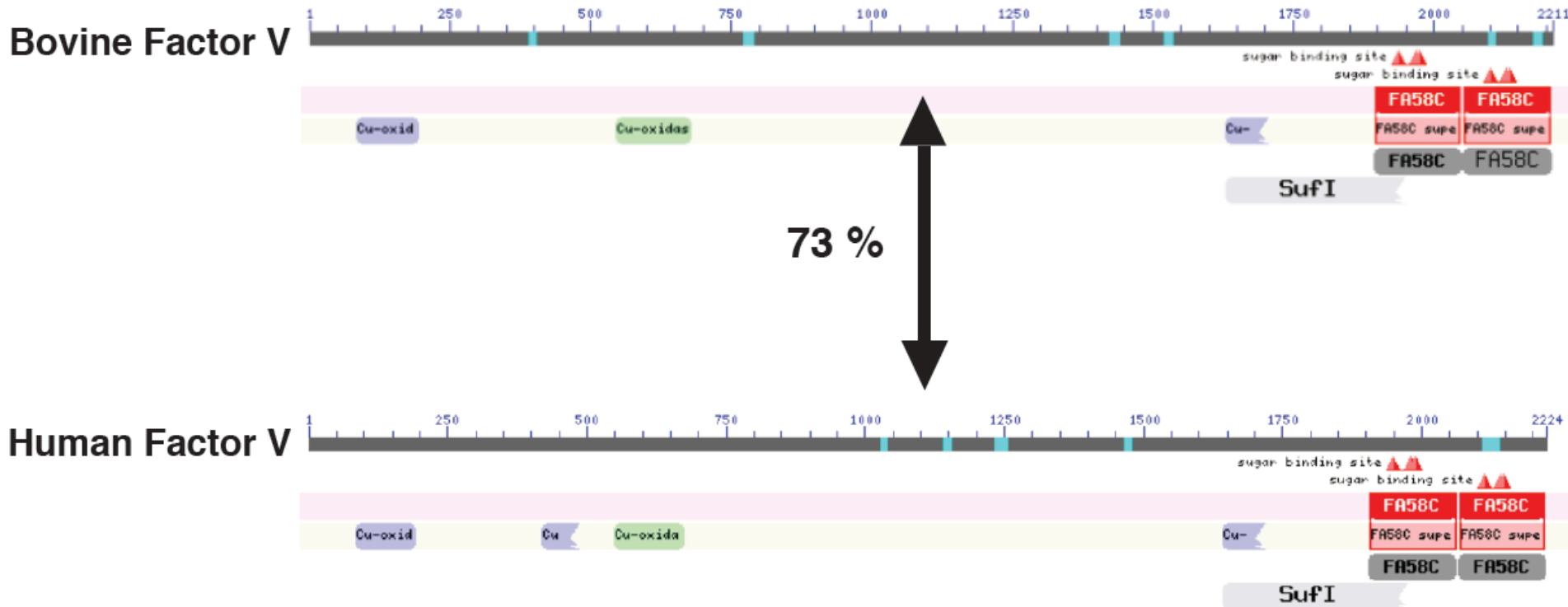
**Potential Immunogenicity**  
Epitope spread extends to self-  
determinants?  
Provision of T cell help activates auto-  
reactive B cells to produce  
autoantibody?

**+**

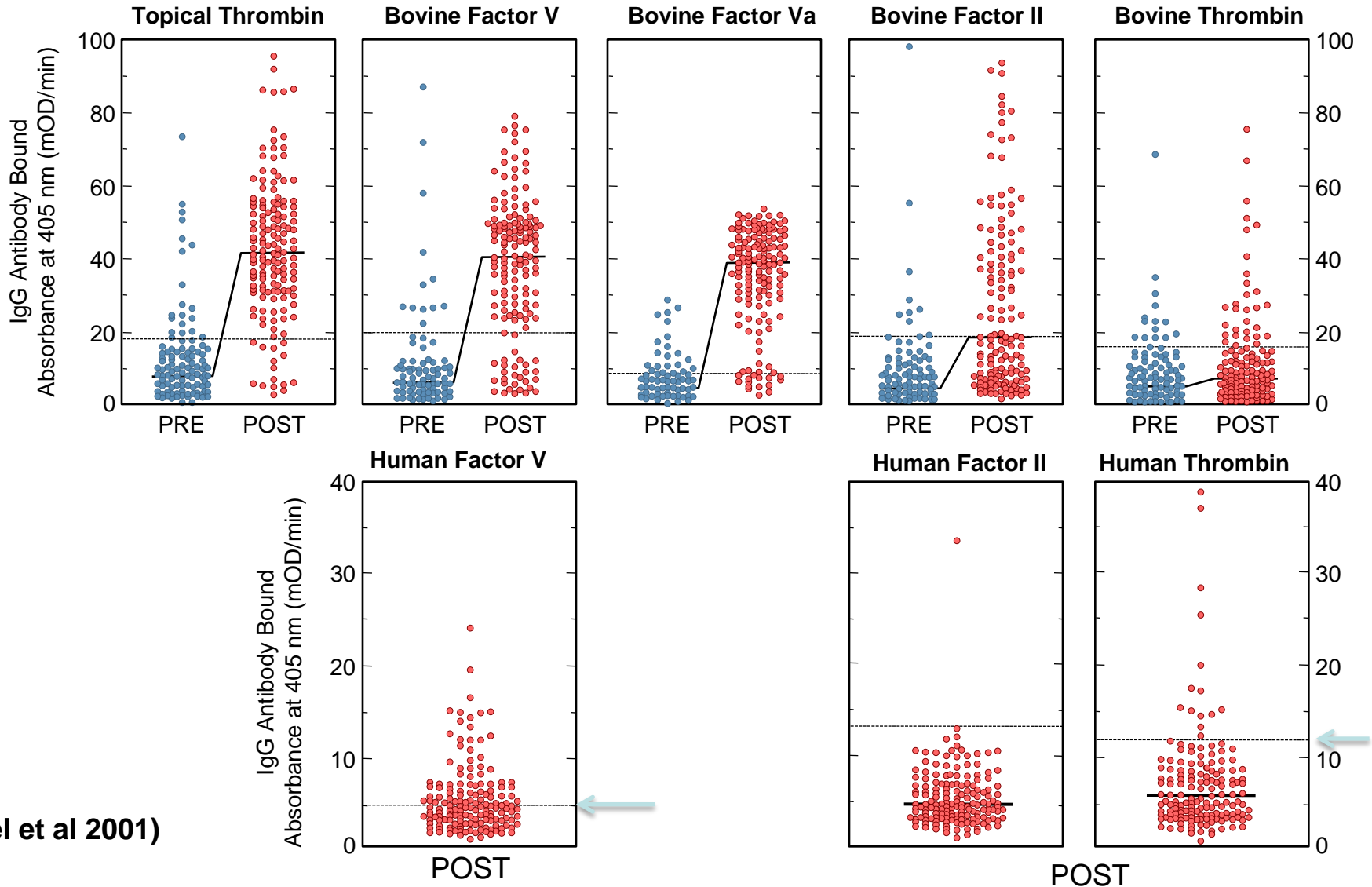
## T Cell Help for Foreign Antigen Provides help to Autoreactive B Cells: Break Self Tolerance



# Bovine and Human Factor V Protein Sequences are Highly Conserved but Have Significant Differences: How Significant to the Immune System?



# Immune Responses to Bovine Factor V and Thrombin Break Tolerance to Respective Human Coagulation Factors



(Ortel et al 2001)



## Cross Reactivity of Immune Response to a Non-Human Therapeutic Protein to a Human Protein with Significant Homology

<b>Injection Number</b>	<b>%Binding and NABs to therapeutic</b>	<b>Cross Reactivity on Endogenous Human Homolog</b>
1 <sup>st</sup> treatment	%; titer; NAB	Initial cross reactivity assessment; NAB to human homolog?
Subsequent treatment	%; titer; NAB	Assess for cross reactivity and NABs to human homolog; consider epitope mapping

## Evaluation and Management of Risk: Foreign Proteins Should be Evaluated for Domains Conserved in Evolution

### • Recommendations

- Perform homology searches for all foreign proteins intended for therapeutic use; define and evaluate extent of homology to human proteins
- Evaluate potential consequences of cross reactive antibodies to human proteins in human autoimmunity and knock out/hyperimmunized mice when evolutionarily conserved;
- Evaluate antibodies to human homologous determinants during and following study;
  - Cross reactivity on human homologous protein; epitope mapping
  - immune response in long term chronic or intermittent use especially important in epitope spread
- *Cautious dose/dosing escalation, changes in route of administration, and exposure of new patient populations*



## Guidance for Writing Guidance

“Those who do not learn from history are doomed to repeat  
it”

George Santayana

“The Generals are always fighting the last war”

Military Dictum

“...and what never frees us from the cost of knowledge,  
which is to act on what we know again and again”

Marge Piercy, American poet



# Acknowledgements

- Daniela Verthelyi, Susan Kirshner, Frederick Mills, Marjorie Shapiro, Steven Kozlowski, OBP, FDA
- Robert Levin, Marsha Reichman, Office of Pharmacovigilance and Epidemiology
- Priya Kishnani, Duke University



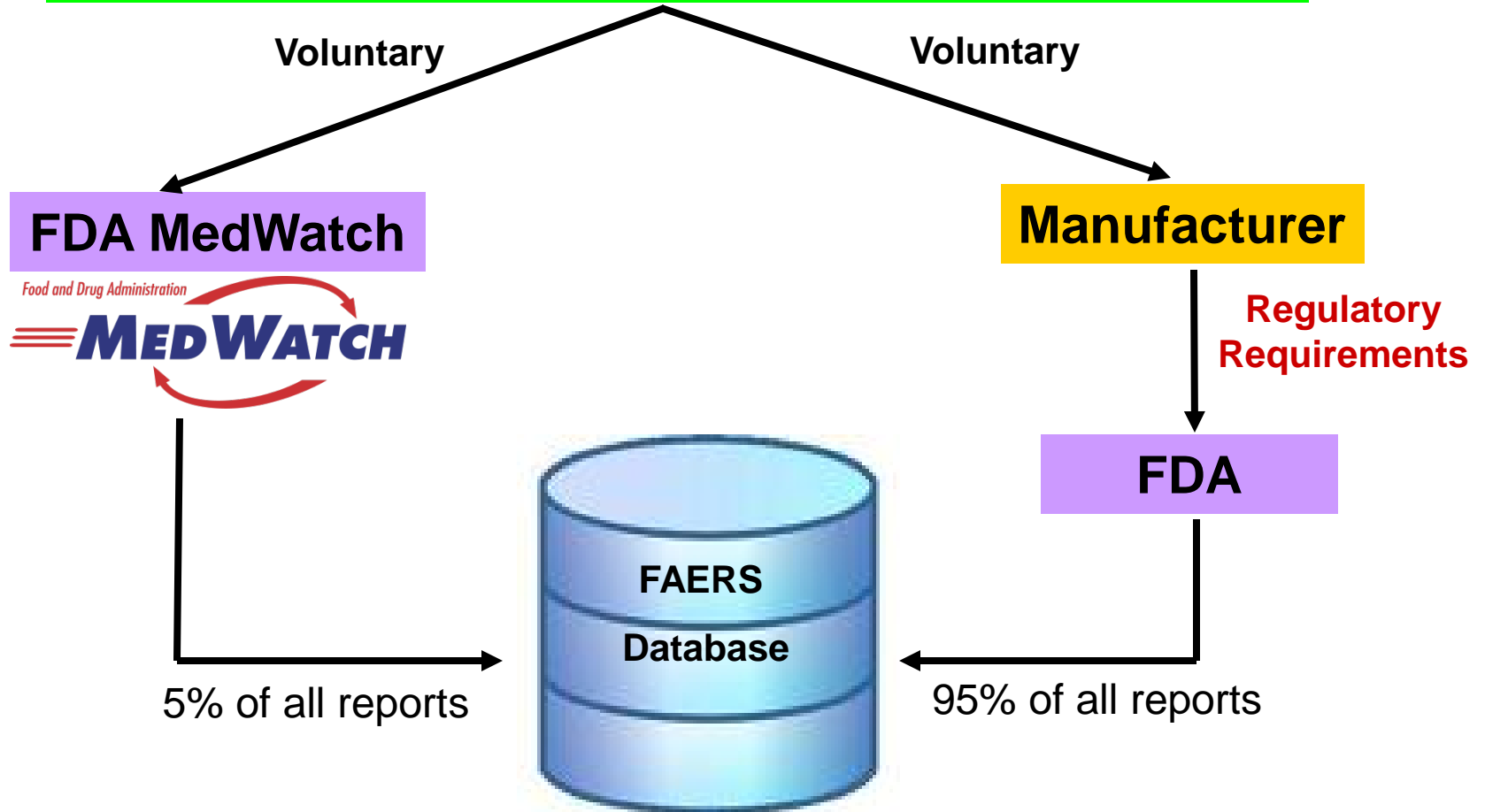
## Challenge Question #2

Which of the following countries does not require practitioners to report adverse events to a national registry?

- A. France
- B. Norway
- C. Sweden
- D. US

# How Postmarketing Reports Get to FDA

Patients, consumer, and healthcare professionals



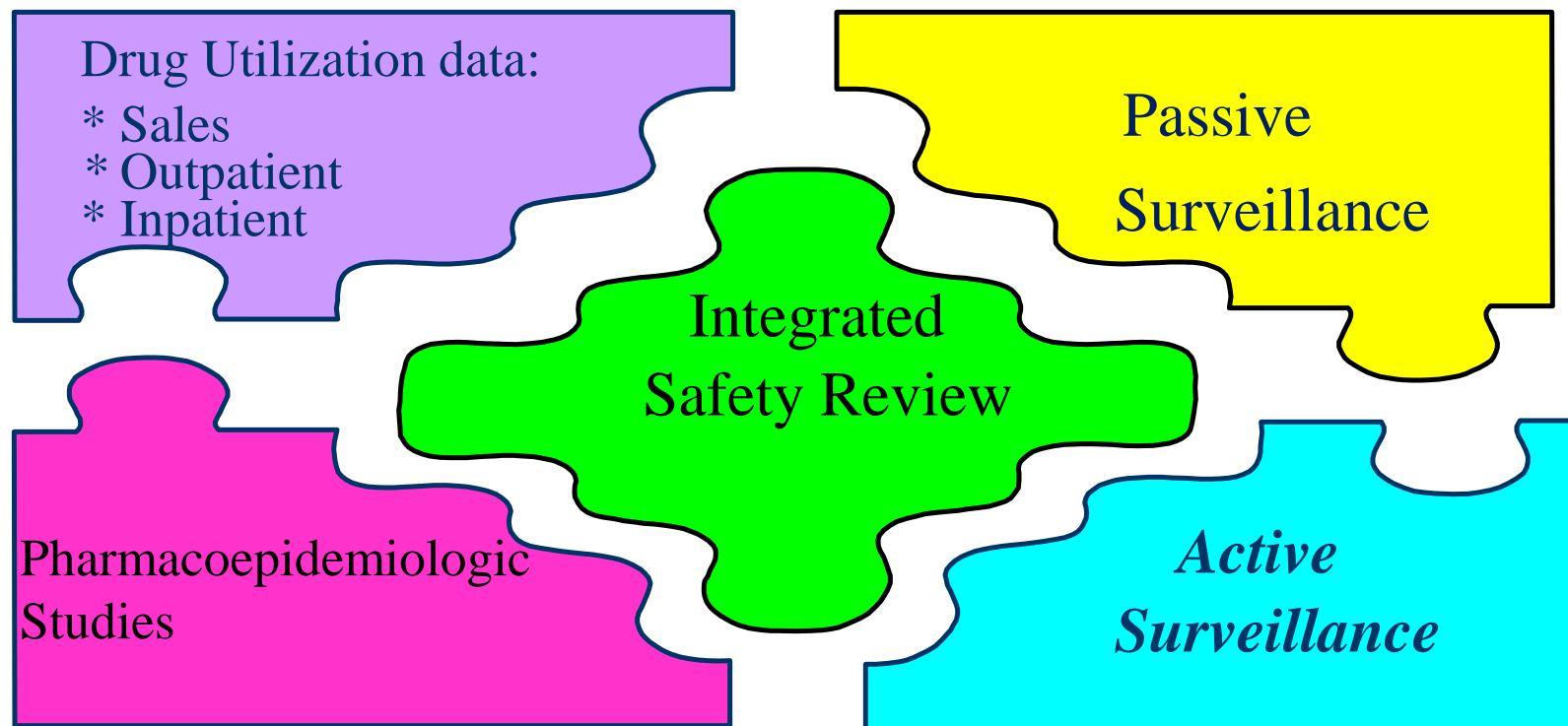
## Types of Postmarketing Surveillance

- Spontaneous/voluntary reporting of cases
  - National (FDA MedWatch)
  - Local or Regional (Joint Commission Requirement)
  - Scientific literature publications
- Postmarketing studies (voluntary or required)
  - Observational studies (including automated healthcare databases)
  - Randomized clinical trials
- *Active surveillance*
  - Drug-Induced Liver Injury Network (DILIN)
  - Sentinel initiative

## Postmarketing safety reporting requirements

- Under 21 CFR 314.80 postmarketing safety reports must be submitted to the agency for the following:
  - **15-day Alert reports: Serious and unexpected** adverse experience from all sources (domestic and foreign)
  - **Periodic Adverse Events Reports:** Domestic spontaneous adverse events that are:
    - Serious and expected
    - Non-serious and unexpected
    - Non-serious and expected
    - Quarterly for the first 3 years then annually

# Components of a Comprehensive Post-marketing Surveillance Program at CDER



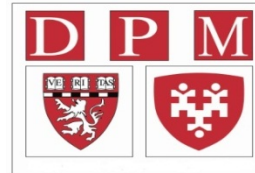
## FDA Sentinel Initiative - Goals

- Develop a national electronic safety monitoring system
  - Leveraging multiple sources of currently available electronic data
  - By partnering with data holders
    - Healthcare systems, insurance companies, etc
  
- Enhance active post-market monitoring of medical product safety
  - More effectively look at common outcomes (e.g. MI, fractures)
  - Have denominators to easily calculate rates
  - Increase sample size with improved access to population subgroups
  
- Use validated design and statistical methods
  
- Near real-time monitoring by using a
  - Common data model & “Library” of tools/resources
  
- Integrate active surveillance with current post-market safety monitoring systems



# Mini-Sentinel Partner Organizations

Lead – Harvard Pilgrim Health Care Ins



Data and scientific partners



Scientific partners



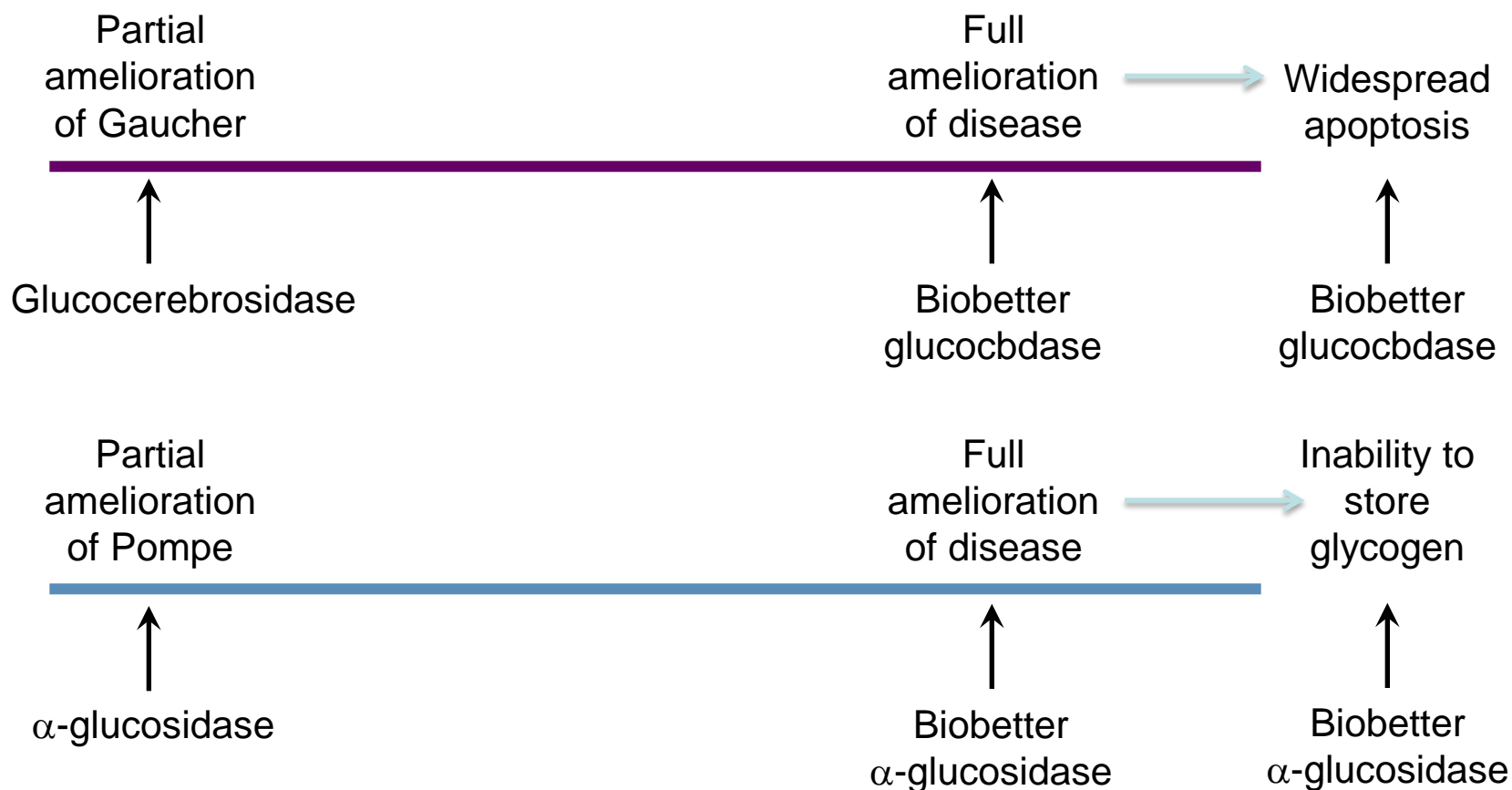
## FDA Sentinel Initiative: Mini-Sentinel Pilot

- Contract awarded: Sept 2009 to Harvard Pilgrim Health Care
  - 18 Data Partners
- Claims and administrative data
  - Assessments depend on **defining exposures and outcomes through codes** (drugs - NDC, diagnoses - ICD-9, procedure codes)
- Approximately 160 million individuals, 2000 - present
  - Different sites have data for different time periods
  - Average length of enrollment (data availability) = 28 months
  - 50 million enrollees currently accumulating data
- Ability to obtain electronic or paper medical records (redacted and de-identified)
  - Not for all enrollees; varies by Data Partner
  - In-patient records much easier than out-patient records



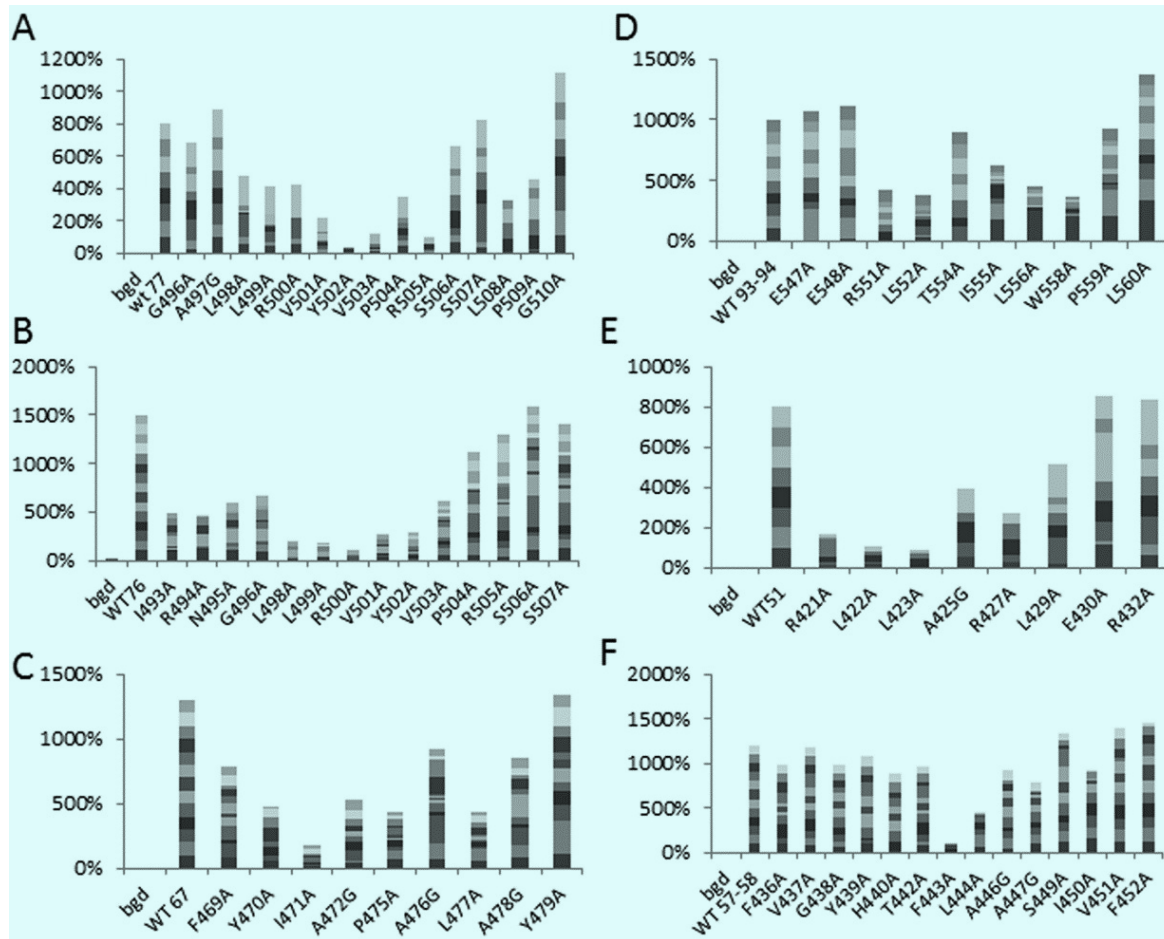
# Supplementary Slides

# Is it Possible to be “Too Biobetter”?

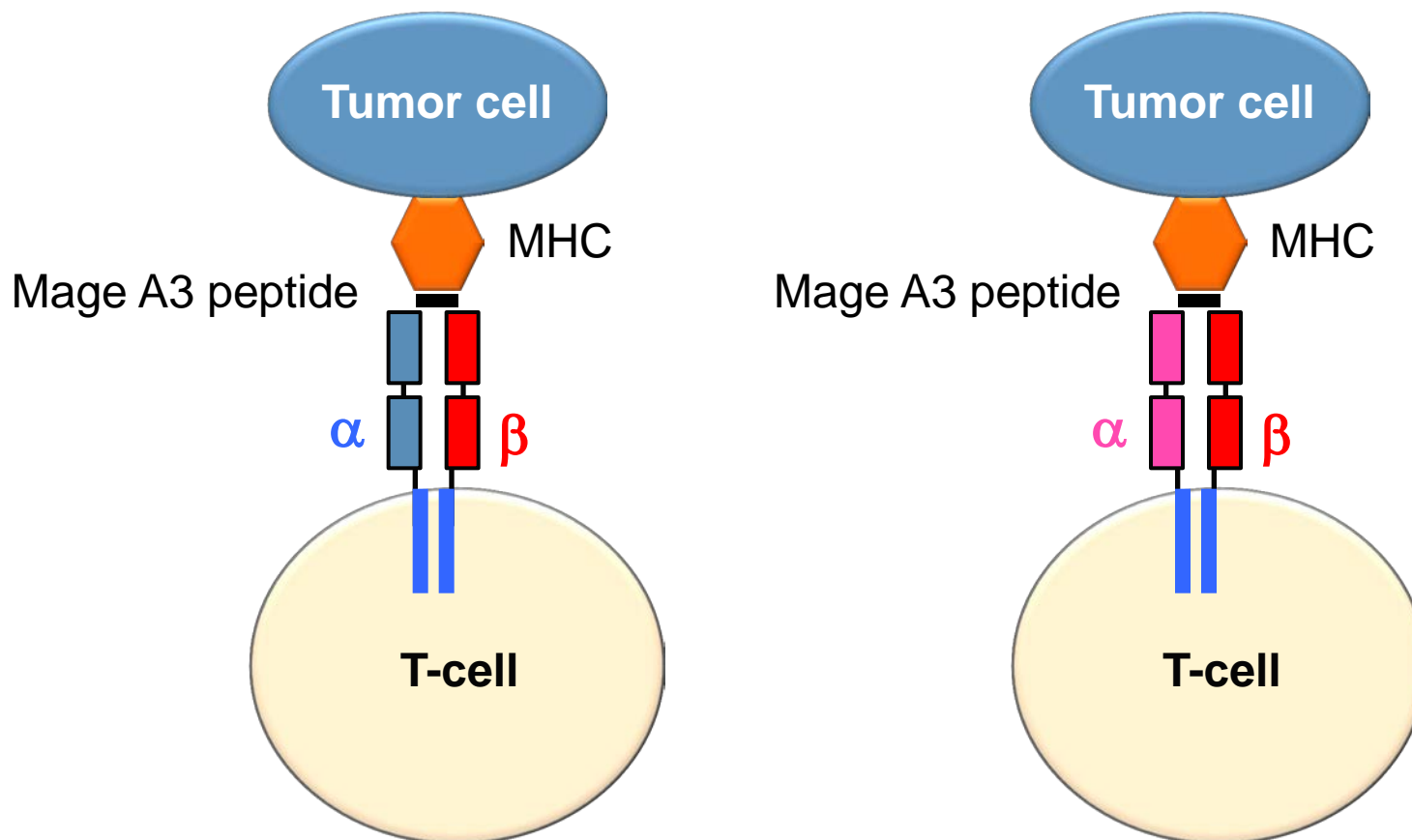


# Introduction of Alanine Substitutions for Defining Critical AAs in T Cell Epitopes in Domain III

(Mazor et al 2014)



## Overriding Thymic/Peripheral Tolerance: Affinity Enhancement of TCR to Tumor Expressed MAGE A3 to Enhance Immunotherapy (Heslop H 2013)



## Avoidance of Cross Reactivity (Hinrichs CS and N Restifo Nat Biotech 2013)

**Table 1. Distribution in healthy tissue of antigens previously ranked highly by NCI as candidate ACT target antigens**

Antigen	NCI priority rank	Gene	Healthy tissue expression that may cause major morbidity <sup>a</sup>
WT1	1	<i>WT1</i>	Kidney, hematopoietic cells <sup>25,62-64</sup>
MUC1	2	<i>MUC1</i>	Lung, liver, pancreas, esophagus, stomach, small bowel, colon, rectum, kidney, bone marrow, lymph node, peripheral nerve, skin, parathyroid gland, adrenal gland <sup>24,25,66</sup>
ERBB2	6	<i>ERBB2</i>	Heart, lung, esophagus, stomach, small bowel, colon, rectum, kidney, urinary bladder <sup>24,25</sup>
<b>MAGEA3</b>	<b>8</b>	<b><i>MAGEA3</i></b>	<b>None<sup>25,91</sup></b>
p53	9	<i>TP53</i>	Bone marrow, spleen, stomach, esophagus, small bowel, colon, rectum, skin <sup>24,25,59</sup>
NY-ESO-1	10	<i>CTAG1B</i>	None <sup>25,91</sup>
PSMA	11	<i>FOLH1</i>	Brain, kidney, liver, spinal cord, nervous tissue, skin <sup>24,25,92</sup>
GD2	12	<i>N/A</i>	Brain, connective tissue from colon and kidney, skin, peripheral nerve, posterior pituitary <sup>72,74,93,94</sup>
CEA	13	<i>CEACAM5</i>	Bone marrow, liver, lung, esophagus, stomach, small bowel, colon, rectum <sup>23-25</sup>
MART1	14	<i>MLANA</i>	Melanocytes including skin, eye, ear <sup>10,24</sup>
gp100	16	<i>PMEL</i>	Melanocytes including skin, eye, ear <sup>10</sup>
Proteinase 3 (PR1)	18	<i>PRTN3</i>	Hematopoietic stem cells <sup>25,95</sup>
Tyrosinase	20	<i>TYR</i>	Melanocytes including skin, eye, ear <sup>24,96</sup>
Survivin	21	<i>BIRC5</i>	Bone marrow, esophagus, stomach, small bowel, colon, rectum, heart, urinary bladder <sup>24,25,97</sup>
PSA	22	<i>KLK3</i>	Pancreas, salivary gland <sup>98,99</sup>
hTERT	23	<i>TERT</i>	Hematopoietic cells, lymphocytes, skin, intestine <sup>100-104</sup>
EphA2	25	<i>EPHA2</i>	Skeletal muscle, liver, colon, lung, esophagus <sup>25,105</sup>

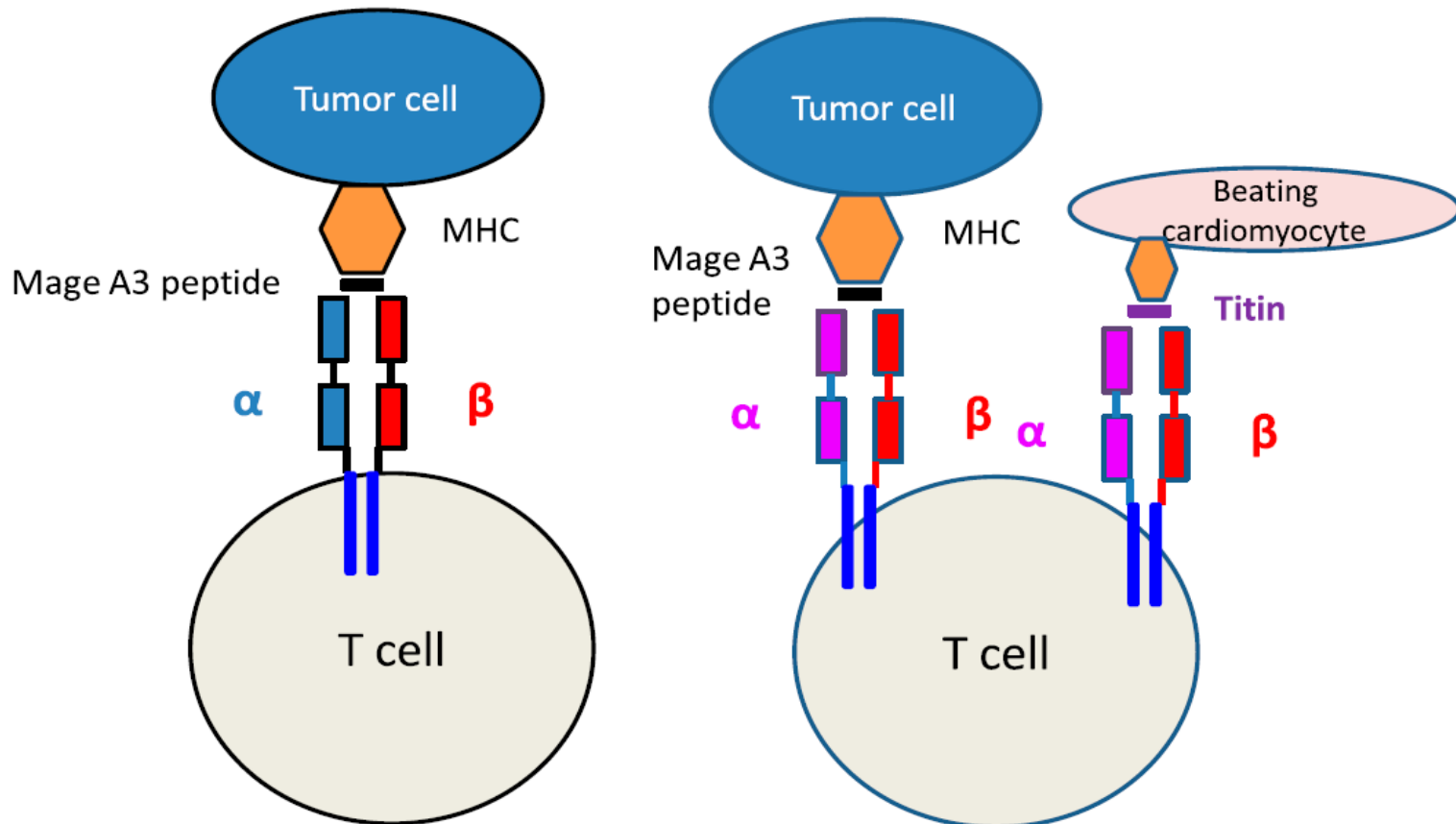
<sup>a</sup>Tissues that might be associated with tolerable toxicities, such as reproductive organs, were not included.  
N/A, not applicable; NCI, National Cancer Institute.

# No Evidence of Cross Reactivity on Healthy Tissues Despite in Silico and in Vivo Preclinical Studies

Cameron BJ et al 2013

- Preclinical studies failed to identify significant cross reactivity on proteins other than MAGE family members: no overt toxicity in HLA-A\*01 transgenic mice
- BLAST for non-MAGE peptides with high level of sequence homology to MAGE A3 and ubiquitous expression: none of 15 peptides were recognized by TCRa3a T cells when pulsed onto HLA-A\*01 APC.

# Affinity Enhancement of TCR to Tumor MAGE A3 has Disastrous Clinical Consequences of Heart Failure and Death in Two Patients (Heslop H 2013)

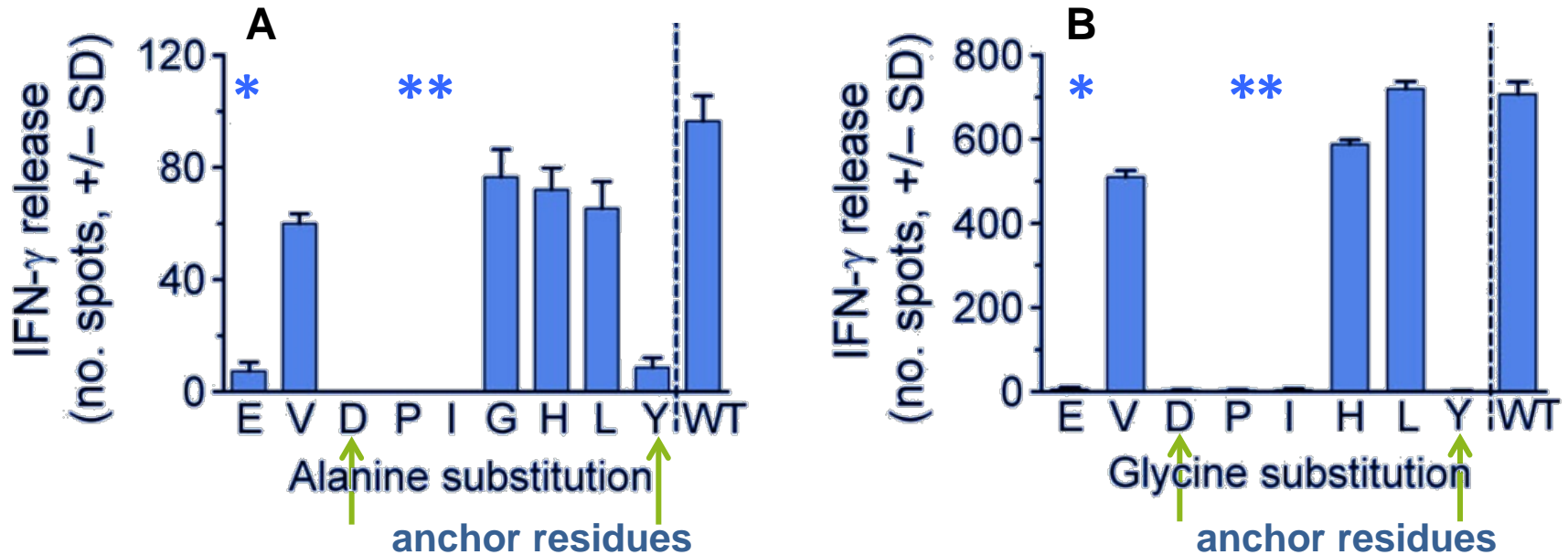


# Post Mortem Analysis: T Cell Activation Against Cardiomyocytes

Cameron BJ et al 2013

- Preclinical studies failed to identify significant cross reactivity on proteins other than MAGE family members: no overt toxicity in HLA-A\*01 transgenic mice
- BLAST for non-MAGE peptides with high level of sequence homology to MAGE A3 and ubiquitous expression: none of 15 peptides were recognized by TCRa3a T cells when pulsed onto HLA-A\*01 APC.
- Post study evaluation found T cell activation against cardiomyocytes from induced pluripotent stem cells but not primary cardiac myocytes
- Highlights critical need for more intensive directed evaluation of TCR cross-reactivity when overriding tolerance mechanisms

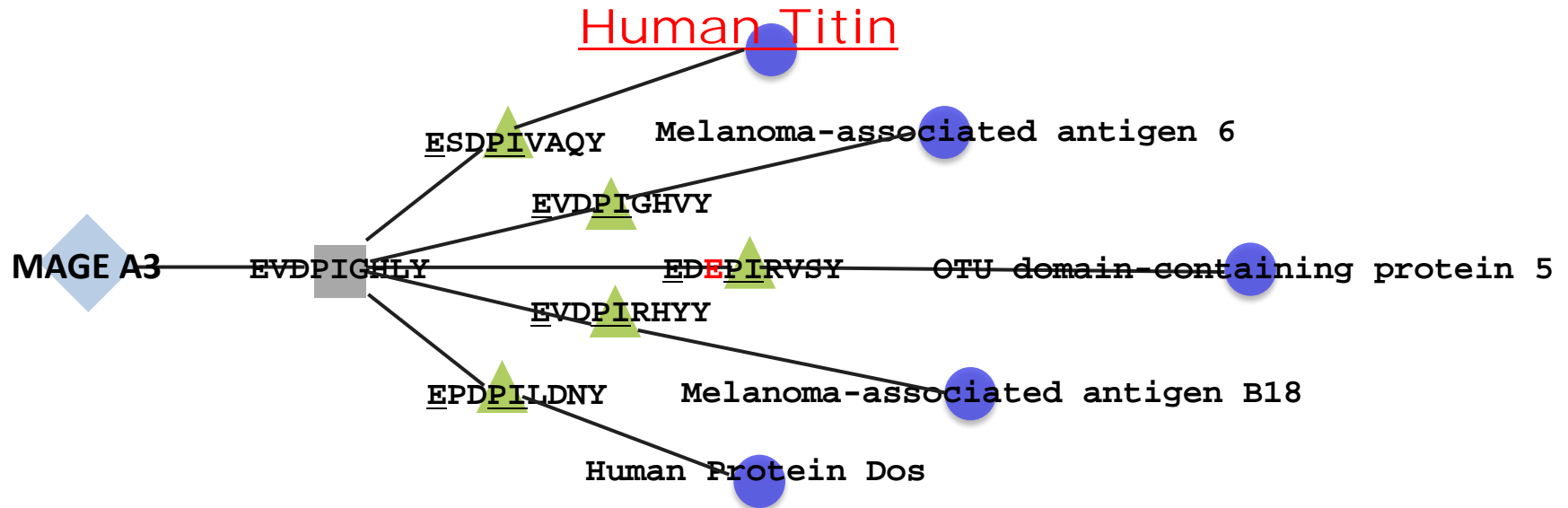
## Key TCR (\*) and HLA Contact Amino Acids in MAGE A3 peptide defined: Cross conservation with other human proteins?



- With immunoinformatics tools, homologous peptides are screened for cross-conservation with human proteins
- A directed in silico search using ScanProsite tool and the “EXDPIXXXY” motif used to identify other proteins containing the motif
- Three proteins identified: titin; nuclease protein from EBV; and ribosomal maturation protein from *C. Difficile*
- Titin is essential in cardiac and skeletal muscle contractility  
(Cameron et al Blood 2014)

## Multiple Cross Reactive Peptides Identified via Immunoinformatics Tool, JanusMatrix

- Melanoma-associated antigen(MAGE A)3: EVDPIGHLY
- Melanoma-associated antigen 6: EVDPIGHVY
- Melanoma-associated antigen B18: EVDPIRHYY
- Human OTU domain-containing protein 5: EDEPIRVSY
- Human Protein Dos: EPDPILDNY
- Human Titin: ESDPIVAQY





## Patient Factors that Bear on Immunogenicity of Therapeutic Proteins

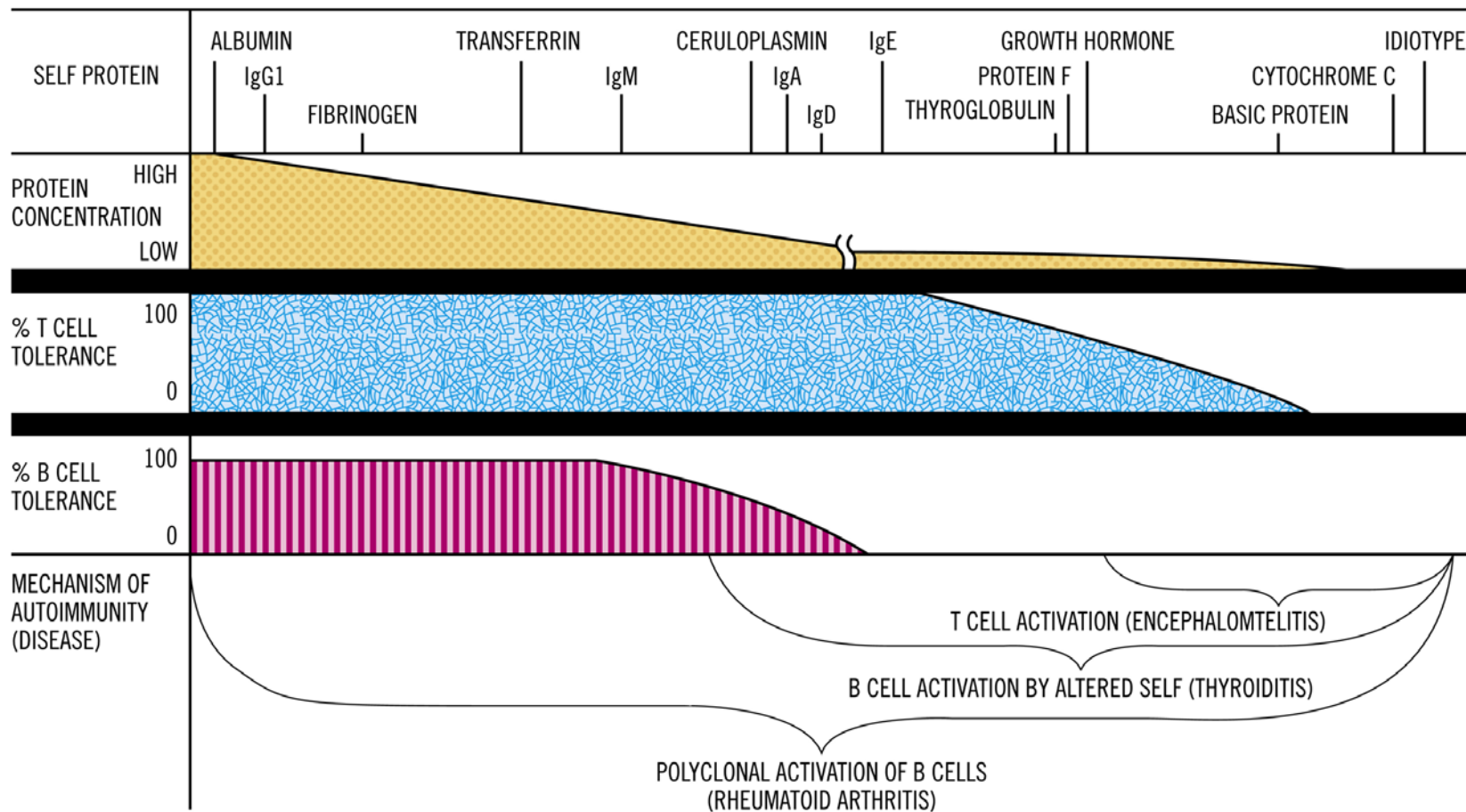
- Immunologic competence
- Dose, frequency, and route of administration
- Genetic factors eg HLA haplotype; polymorphisms in cytokine genes
- Prior sensitization/history of allergy
- *Extent of tolerance to endogenous protein counterpart of therapeutic*

## Product Factors that Bear on Immunogenicity

- Protein origin: foreign vs self-with qualifications
- Protein structure:
  - aggregates
  - post-translational modifications/chemical degradation
- Impurities: with adjuvant properties
- Immunomodulatory properties of the protein therapeutic: immunostimulatory vs immune suppressive

# T Cells More Robustly Tolerant than B Cells to Self Proteins

(Weigle, 1980)



## Mitigation Strategies for Immunogenicity

- Engineer the patient's immune response
  - Immune suppression
  - Immune tolerance induction
  - Appropriate in some, but not all, 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

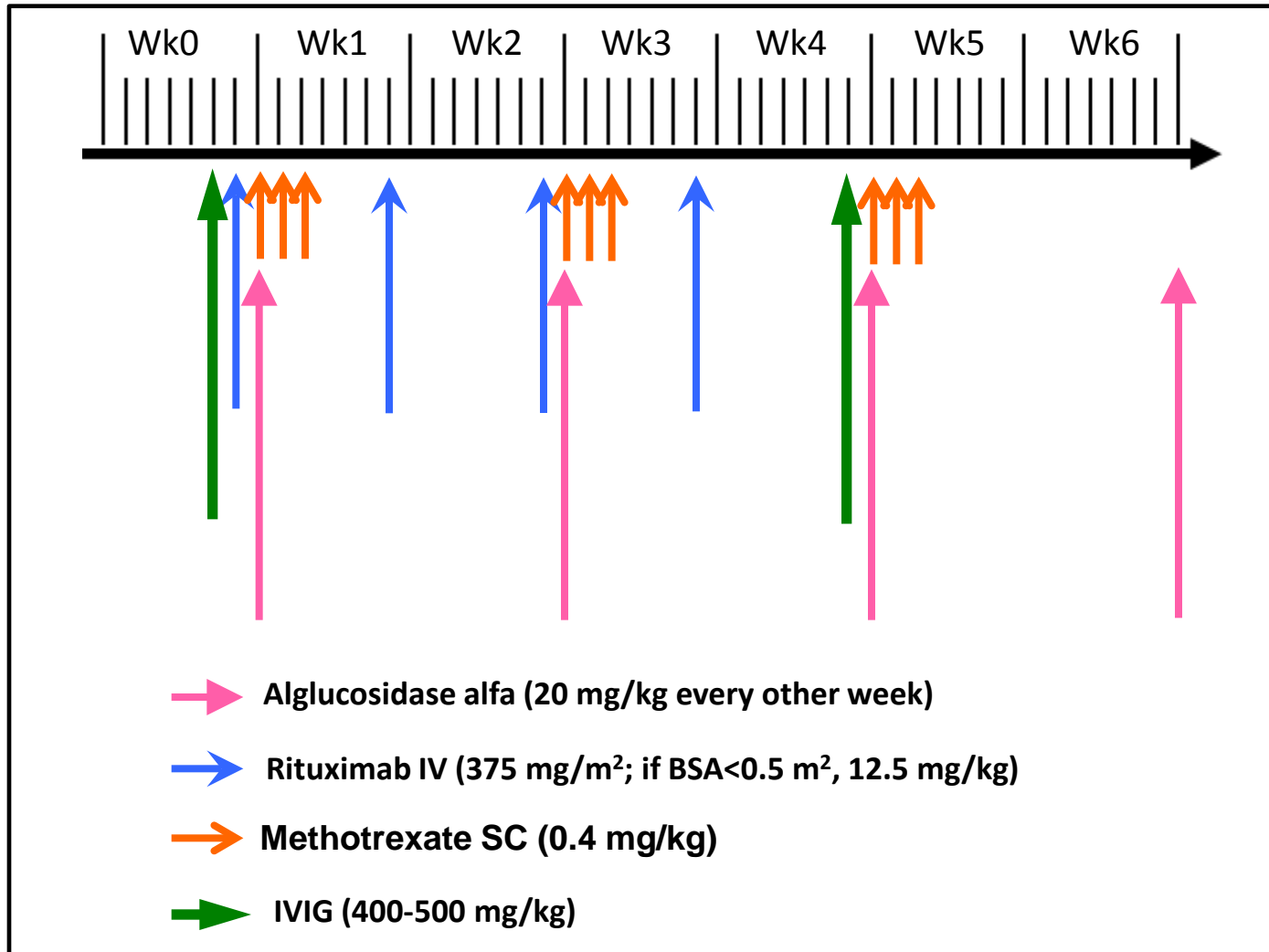


## Mitigation of Epo Related PRCA in Patients: Rigorous Immune Suppression Restores Tolerance

(Bennett et al 2005)

Immune suppression	Dose Range	Route	Observed Recovery
Cytosan Prednisone	50-100mg/d 1mg/kg/day	PO	87%
CsA	100mg bid or 5-8 mg/kg/day	PO	67%
Prednisone	1mg/kg/day	PO	56%
IVIg	2g/kg over 2-5 days	IV	11%
CsA/Tac + Transplant (19)			95%

# Prophylactic ITI Protocol



## **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 less immune suppressive
  - clear tolerance measures;
  - may prevent irreversible tissue damage due to diminished ERT activity.

# 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

