From Assessment of Immune Responses to Therapeutic Proteins to Clinical Challenges: Addressing the Role of the Immune System in Complex Diseases

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Disclosure and Disclaimer

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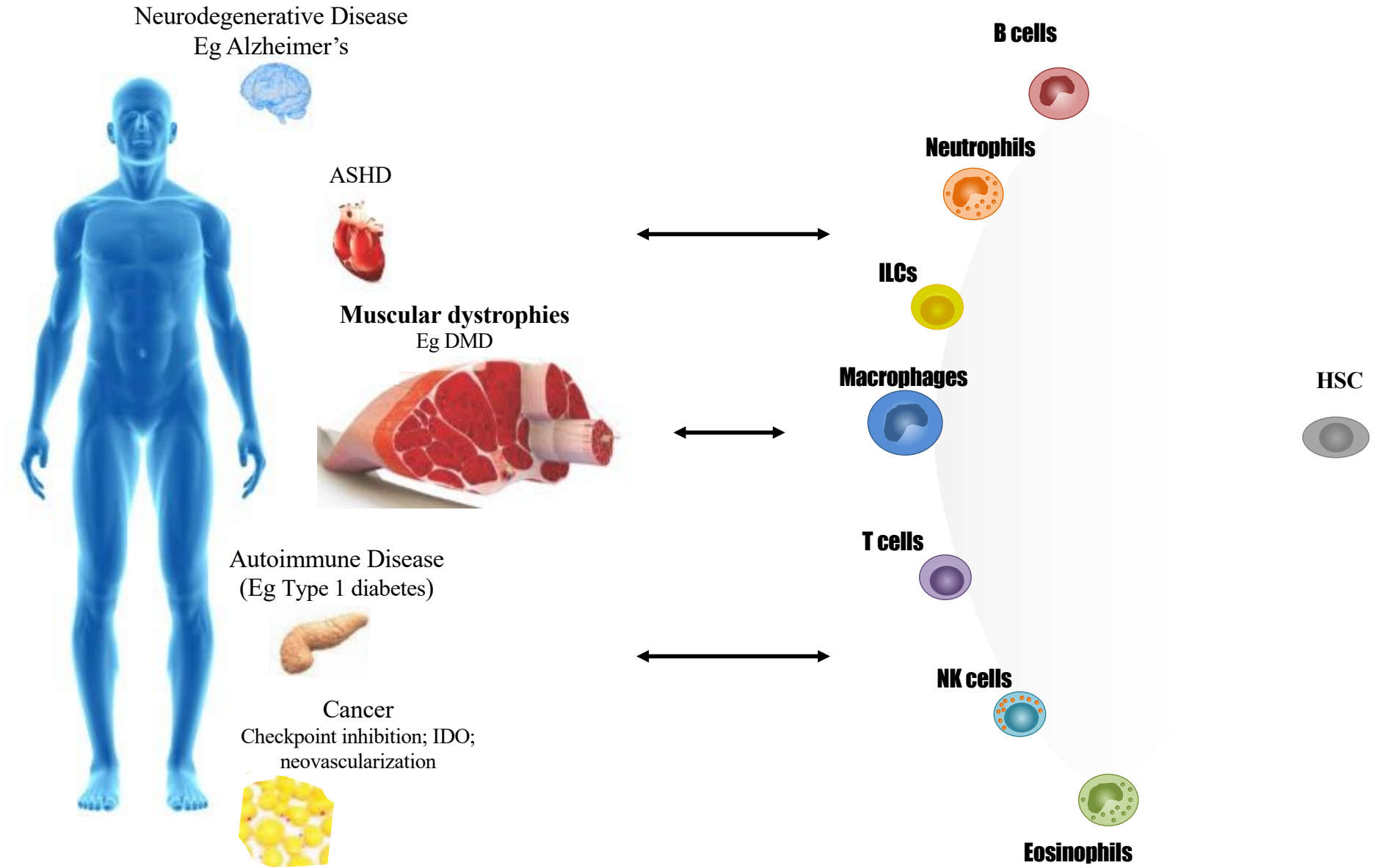
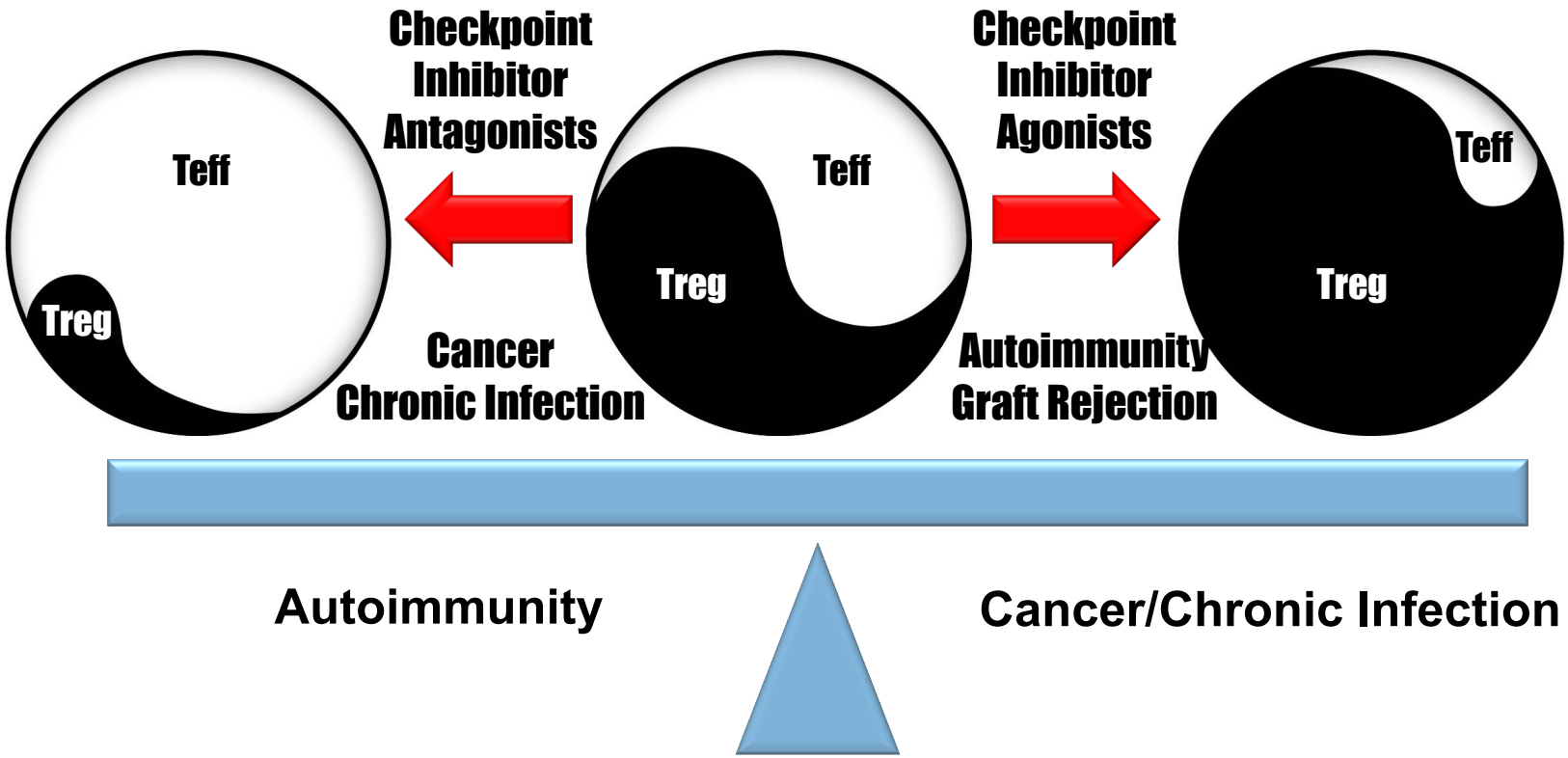
Immune Responses that Mediate or Modulate Human Disease



Manipulation of the Immune System for Therapy of Complex Diseases



U.S. Food and Drug Administration
Protecting and Promoting Public Health
www.fda.gov

Medicine Through Time (Twentieth Century)

Do you know your stuff?

1. **What is a magic bullet?**

   A drug that targets specific bacteria and deosn't affect any other

2. **Who was Salvarsan 606 discovered by and what does it treat?**

   Paul Ehrlich, treat syphilis

3. **When did Domagk have the chance to try out Prontosil on humans?**

   Daughter pricked her finger on an infected needle

3. **Give three illnesses that sulphonamides can treat**

   Meningitis, gonorrhoea and pneumonia

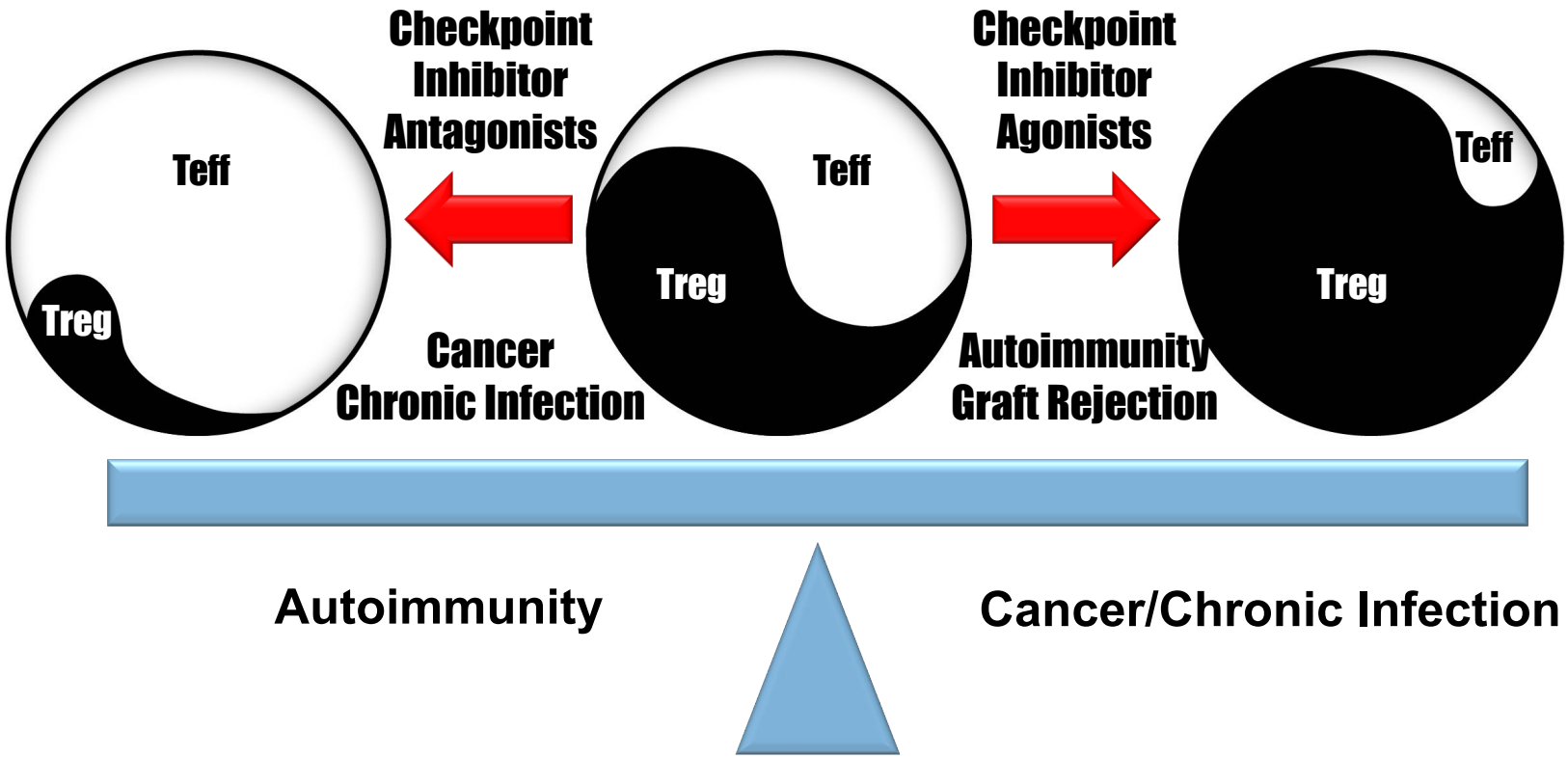
No Magic Bullets for Preventing or Curing Complex Infectious Diseases: Failure to Successfully Address Latent States

- HIV
- EBV
- CMV
- Zika

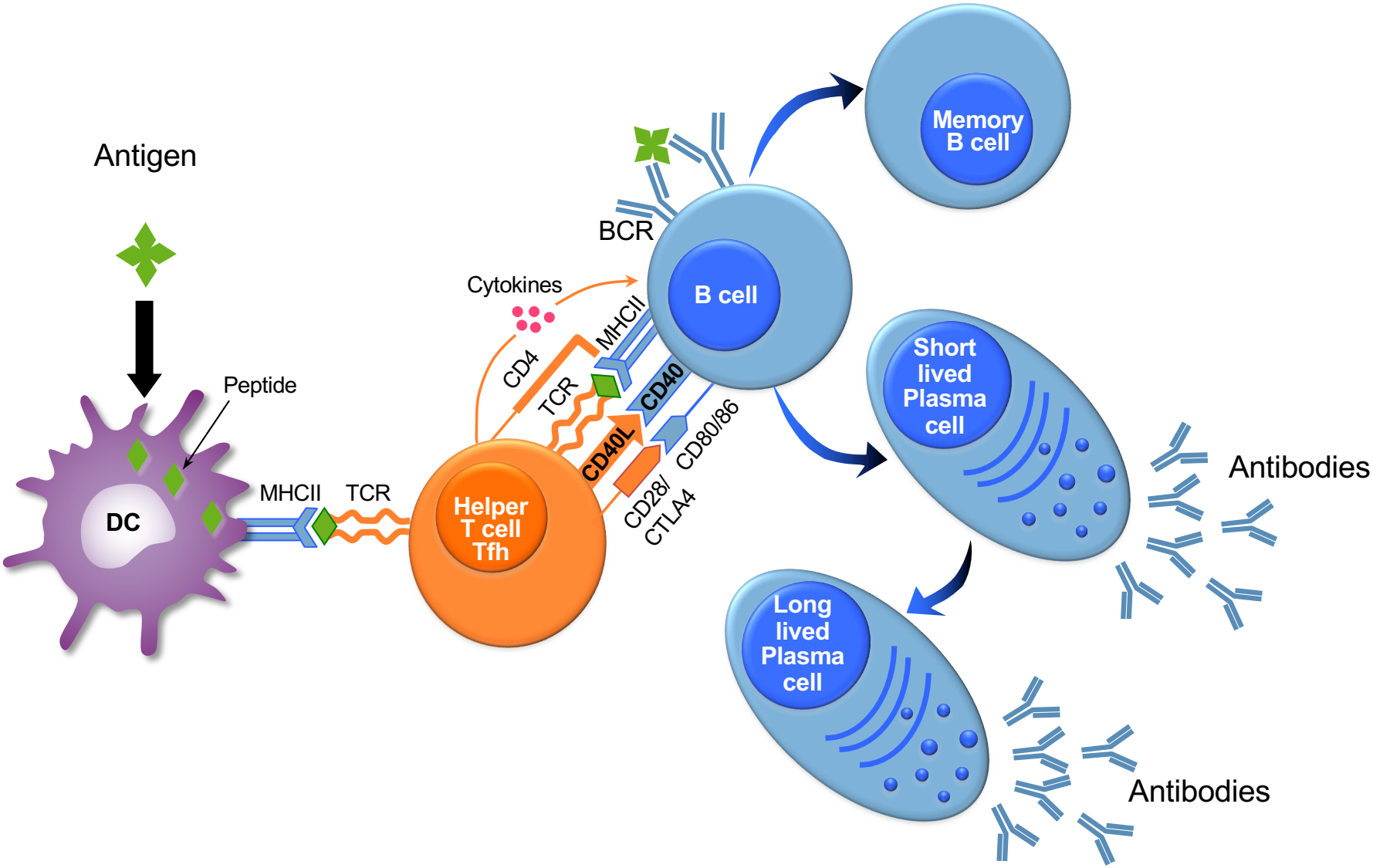
No Magic Bullets for Complex Diseases

- Most solid tissue advanced cancers
- Autoimmune Diseases
- Muscular Dystrophies

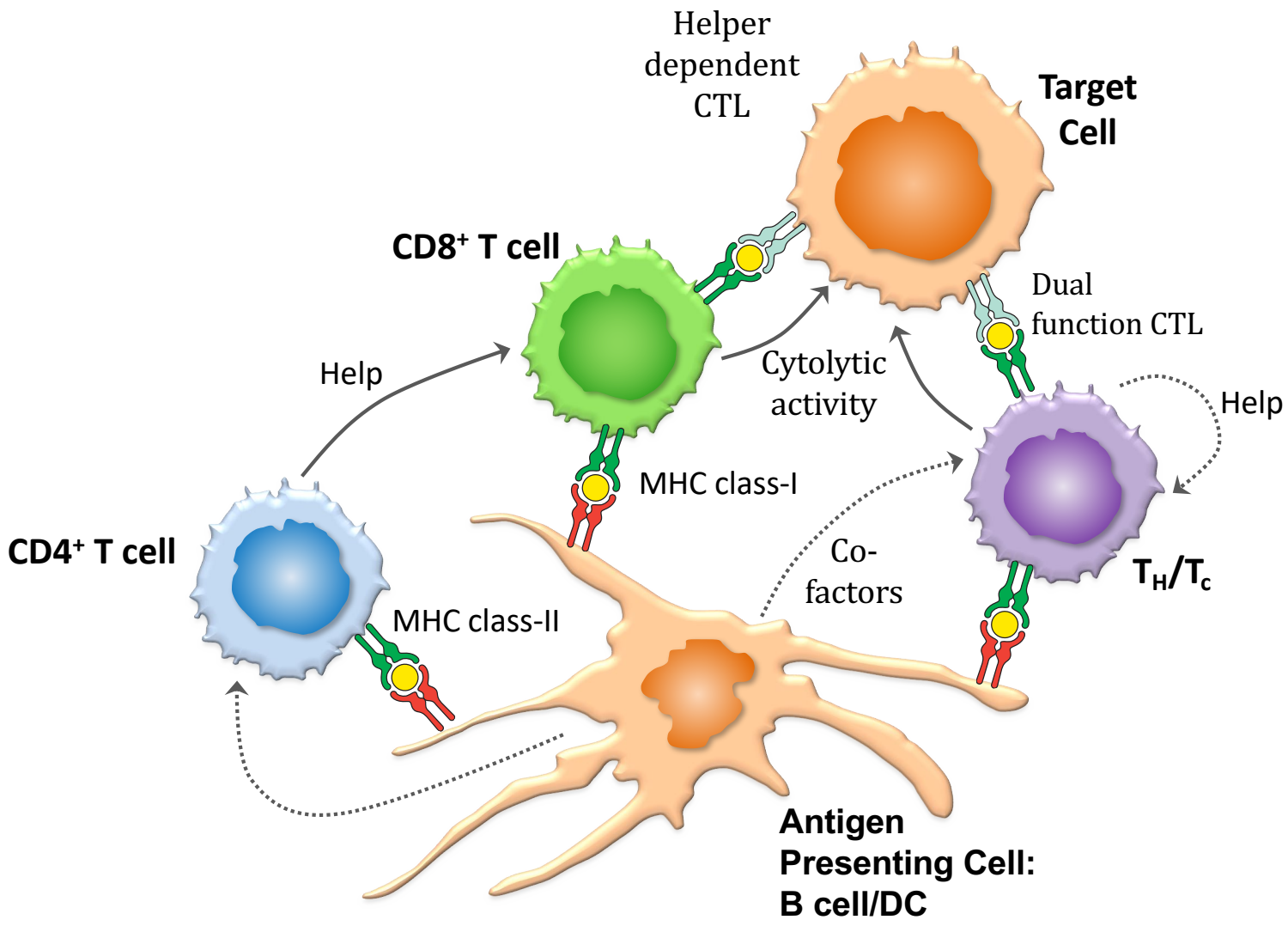
Manipulation of the Immune System for Therapy of Complex Diseases



Thelper Cells are the Lynchpin in Generating Antibody Responses to Protein Therapeutics and Endogenous Proteins

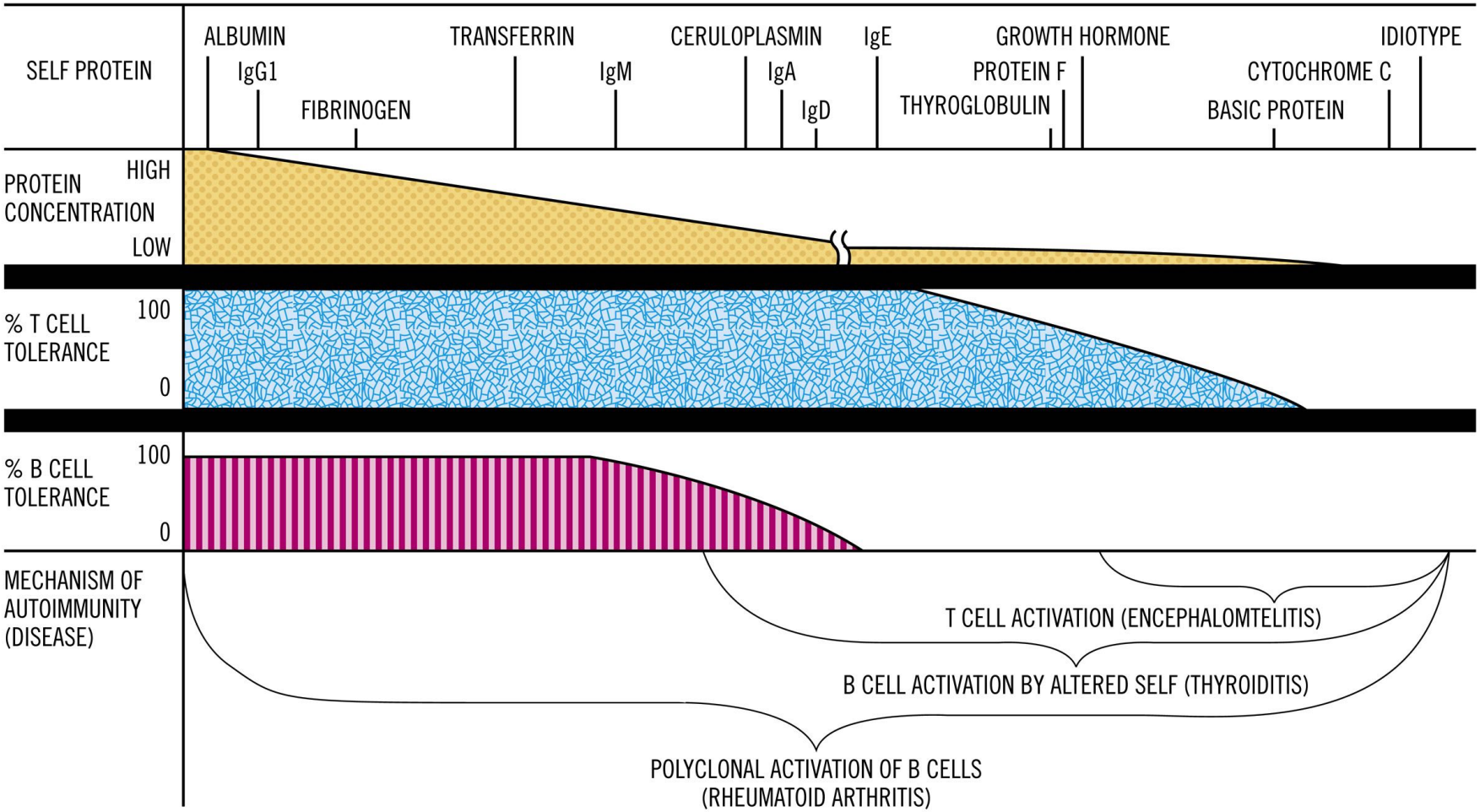


Thelper Cells Critical for Generation of Cytolytic T Cell Responses



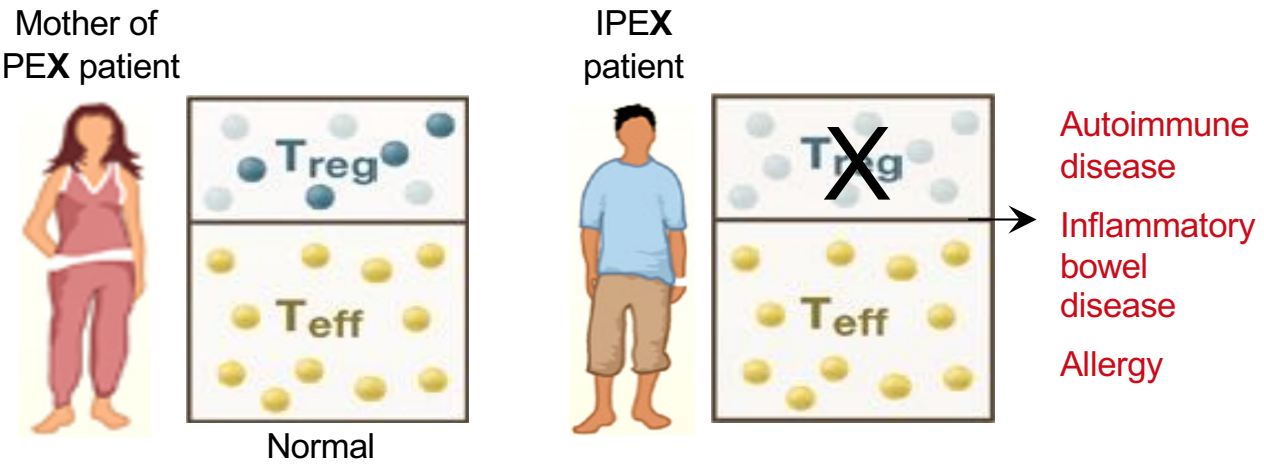
T Cells More Robustly Tolerant than B Cells to Self Proteins

(Weigle, 1980)

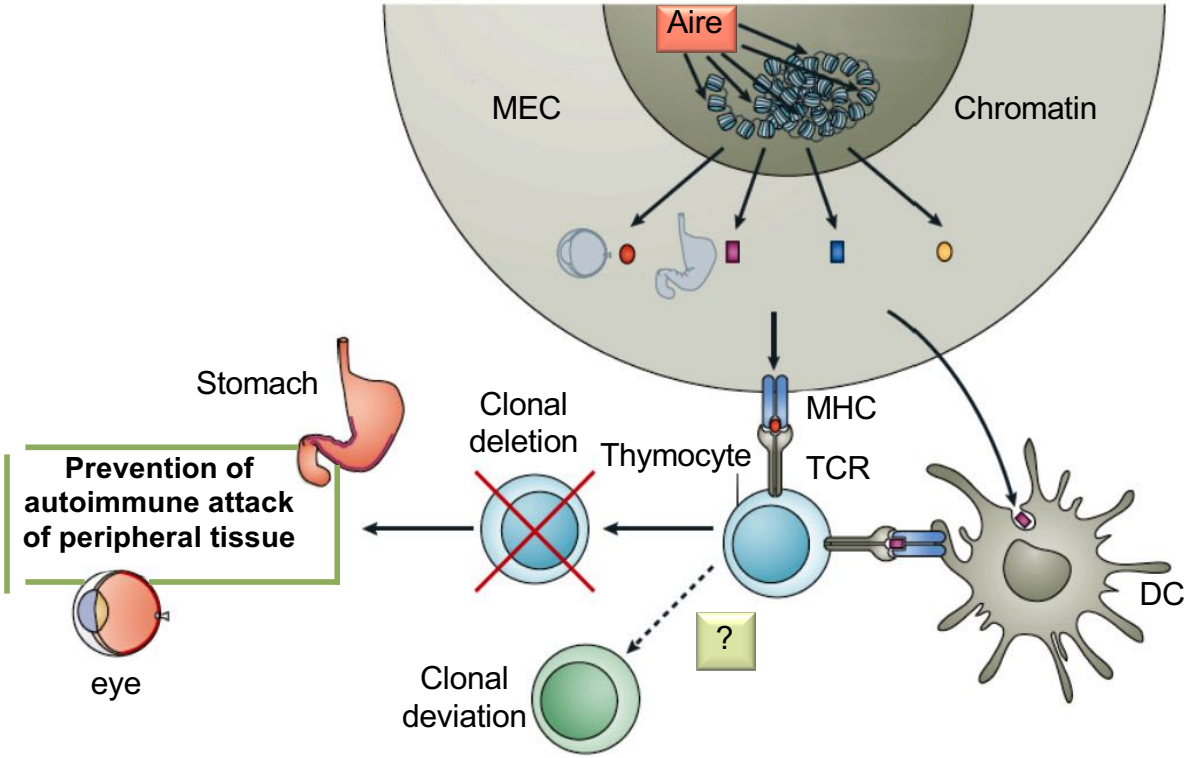


Immune Tolerance Based on Thymic Mechanisms: Clonal Deletion and Tregs

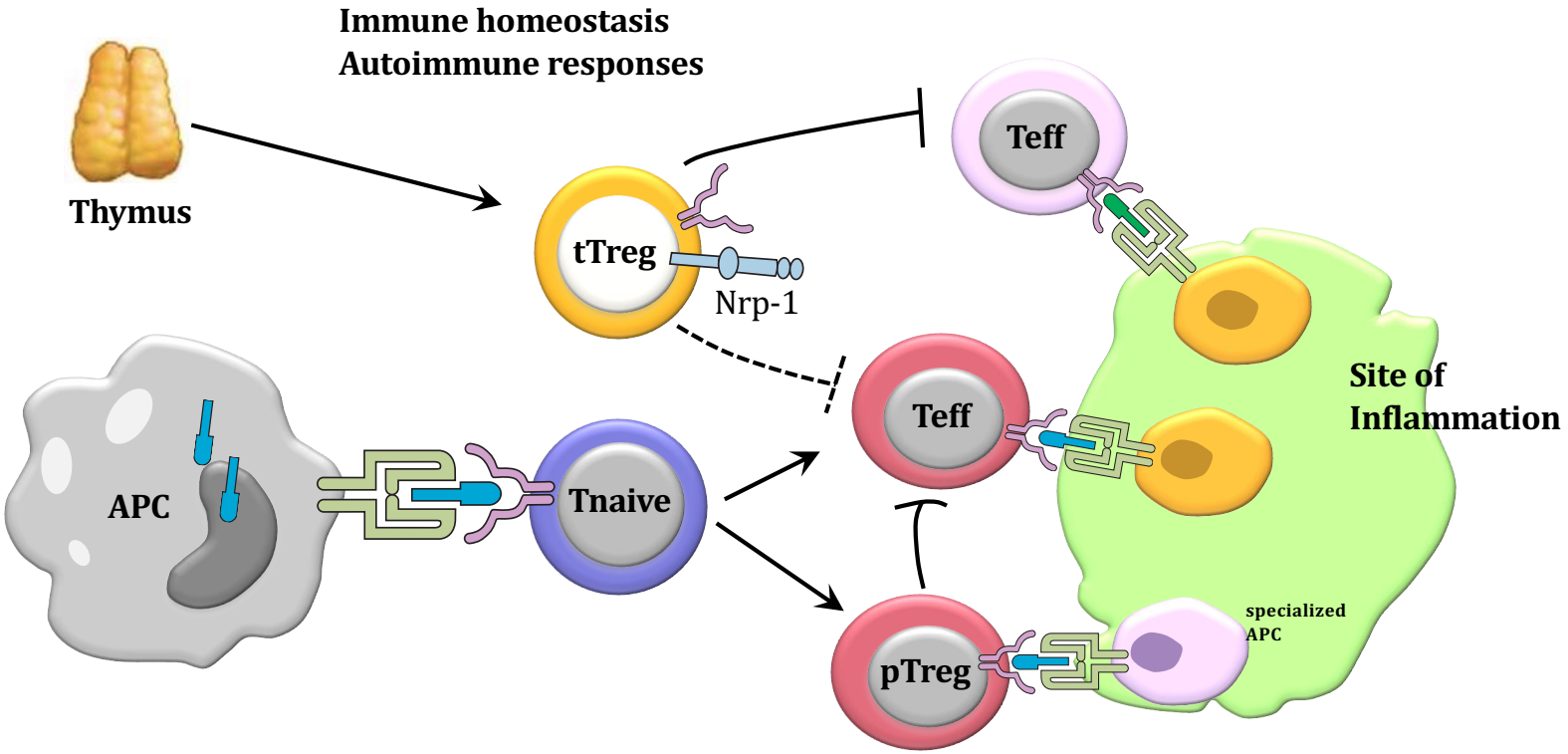
FoxP3+ Tregs Prevent Autoimmunity Mutations in FOXP3 Cause IPEX
(Sakaguchi et al 2008)



Autoimmune Regulator (AIRE) Promotes Expression of Peripheral Tissue Antigens in the Thymus: mutations in AIRE Associated with Autoimmune Polyendocrine Syndrome Type1
(Mathis and Benoist 2007)



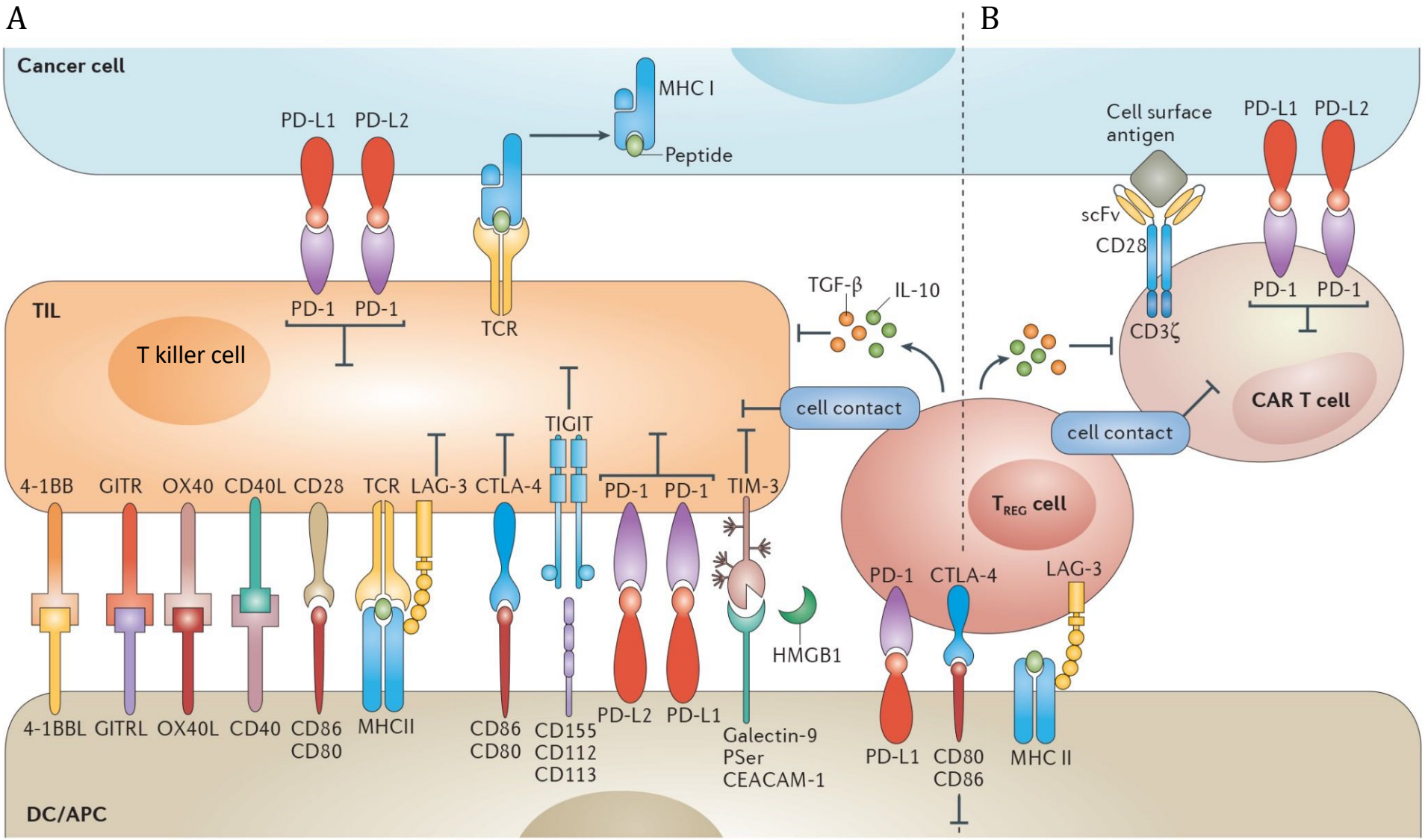
Autoimmunity is Suppressed by both Thymically and Peripherally Generated Tregs



Local immune suppression
Oral tolerance
Fetal tolerance
Mucosal tolerance

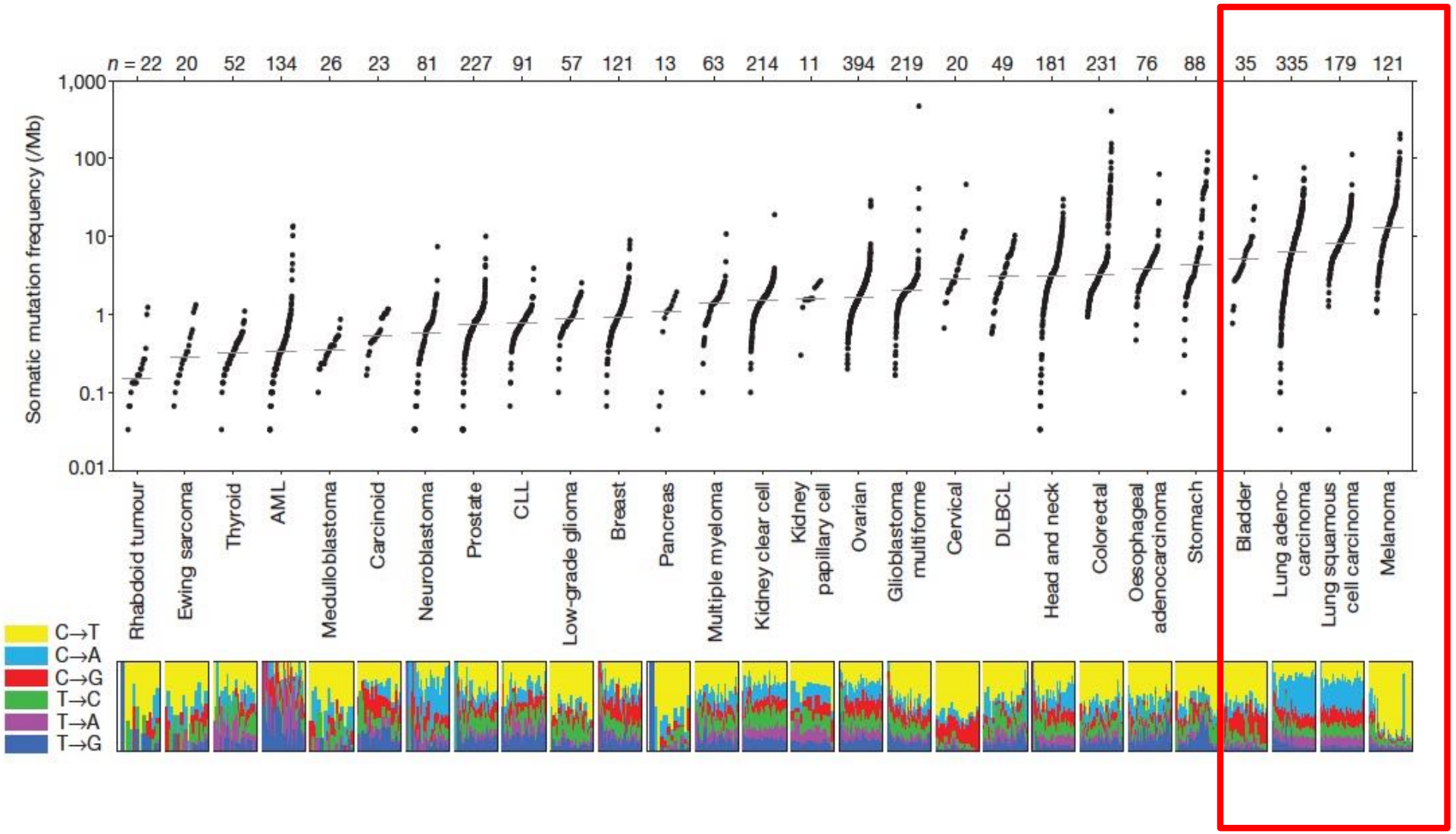
Yadav M et al Frontiers in Immunology 2013

Complexity of the Immune Suppressive Tumor Environment: Cellular and Molecular Mechanisms



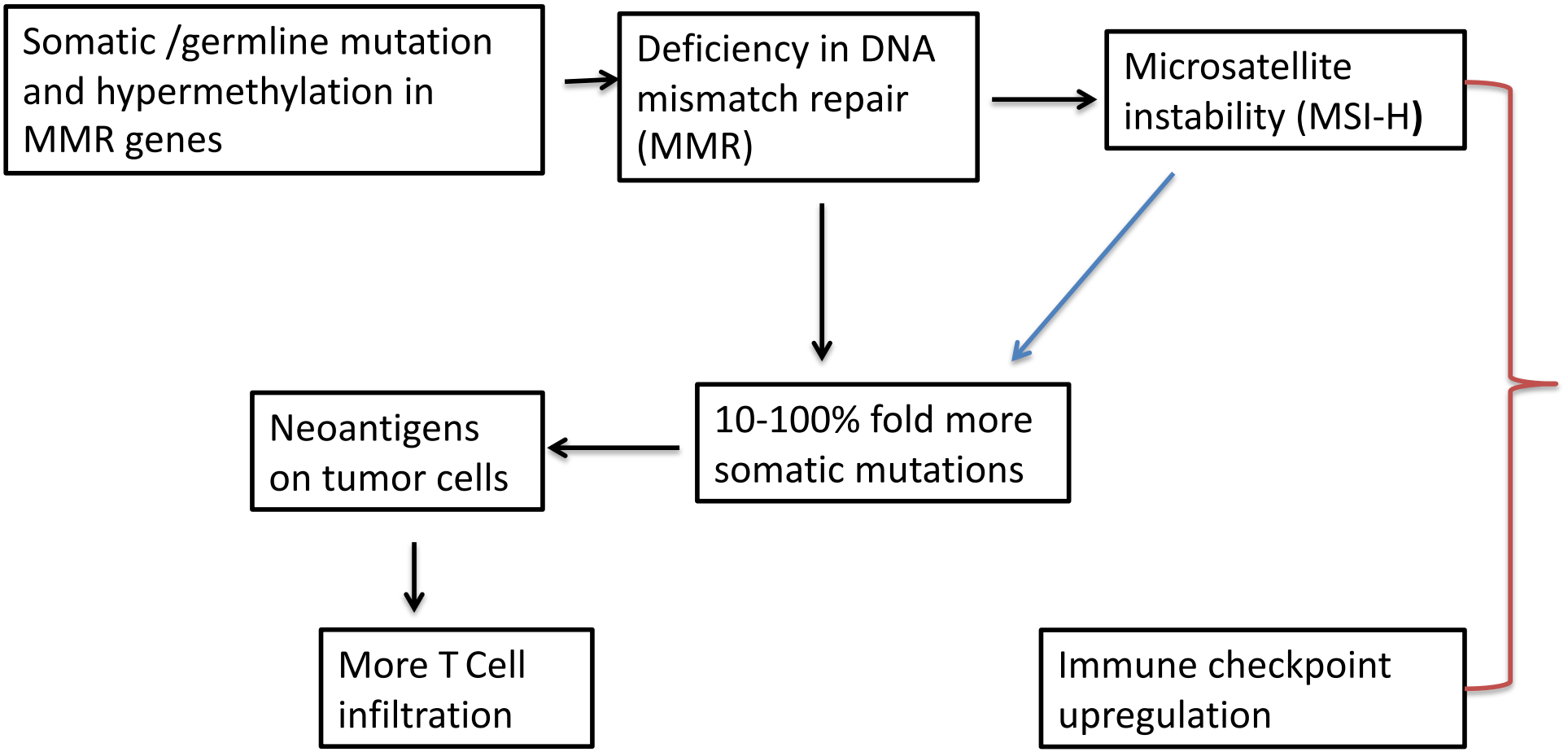
Wolchok J et al 2016 NATURE REVIEWS CLINICAL ONCOLOGY

Tumor Mutational Burden Causing Neoantigen Expression is Key in Tumor Immunogenicity and Clinical Response to Immunomodulation



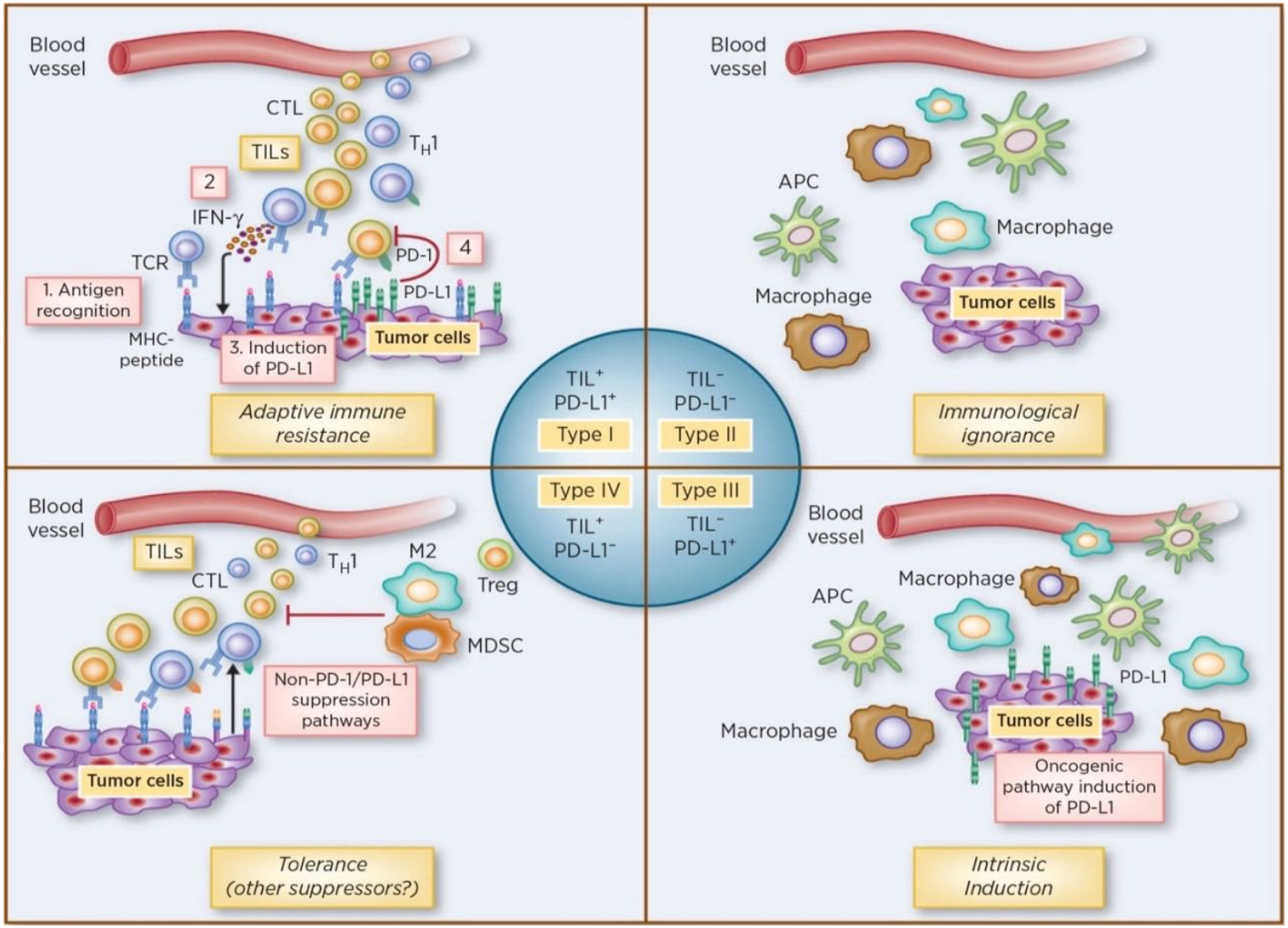
Lawrence MS *et al*, Nature 2013

Immunogenicity Lessons from Tumors with DNA Mismatch Repair Deficiency/ Microsatellite Instability: The Gift that Keeps on Giving



Induced mutations and neoantigen generation through induction of MMR deficiency

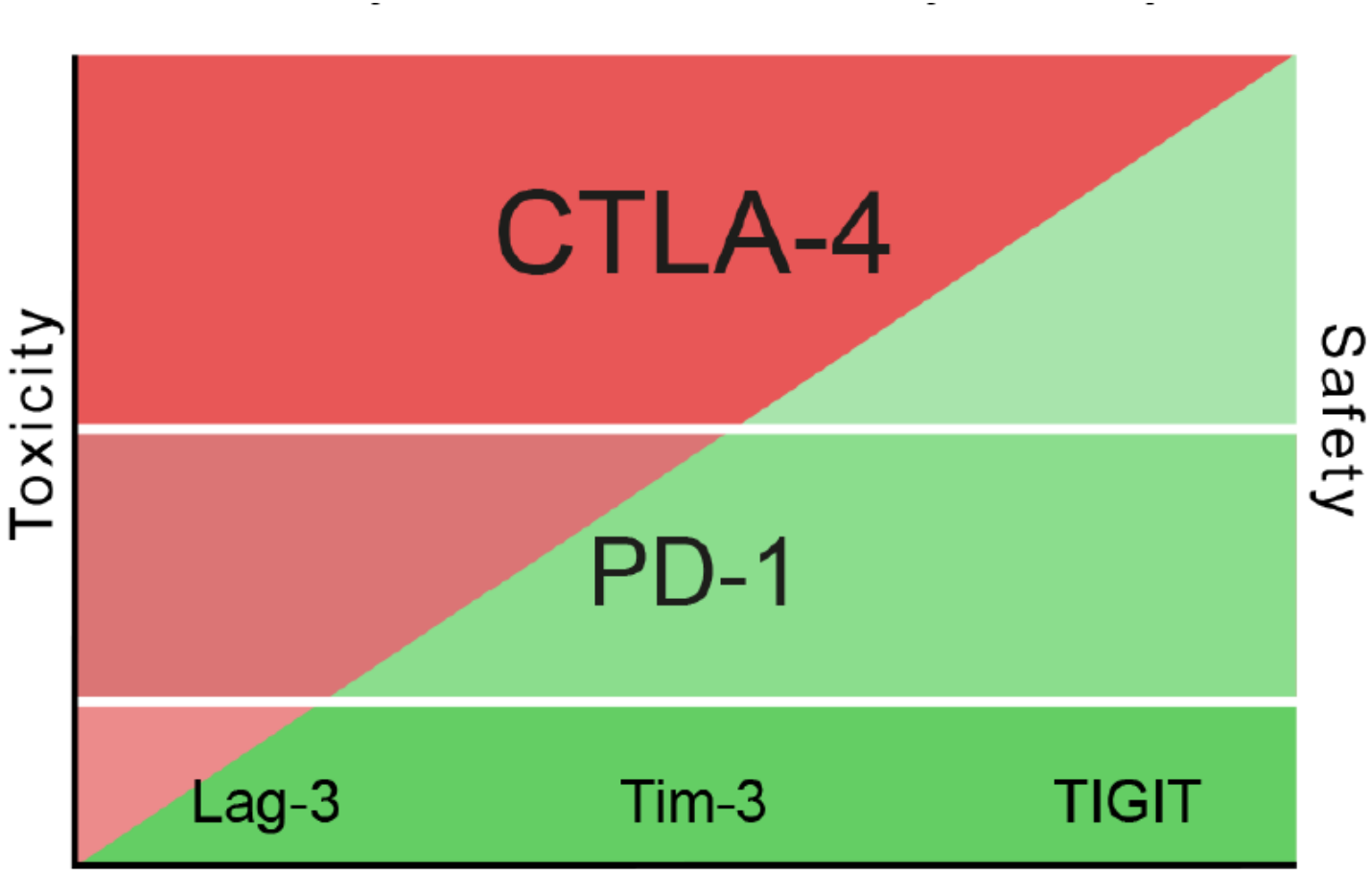
Profiles of Tumor-Immune System Interactions:neoantigens elicit T cell infiltration and upregulation of checkpoint inhibitory molecules



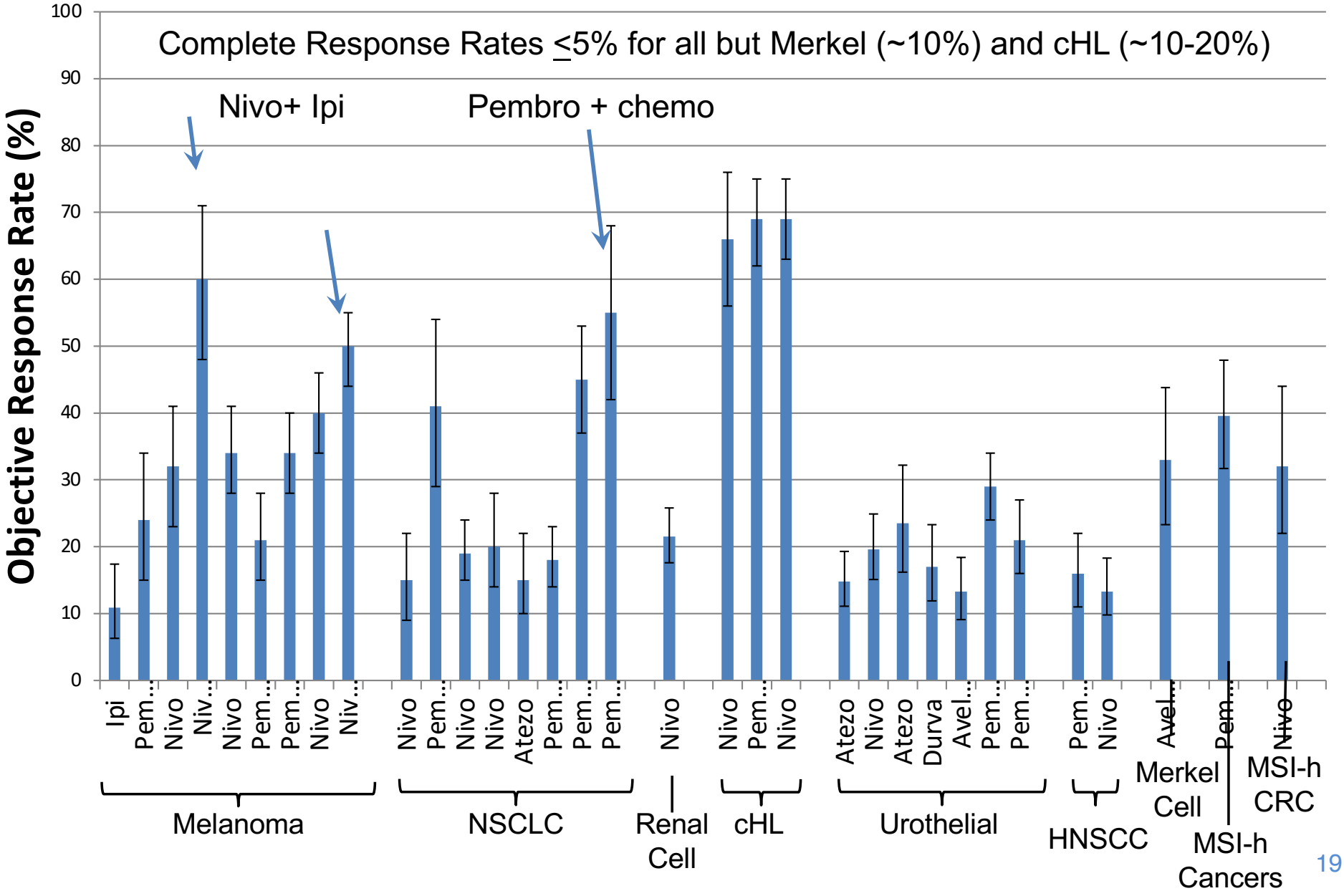
(Smyth M et al 2015)

Hierarchy of Co-Inhibitory Receptors: Impact on Maintenance of Self Tolerance

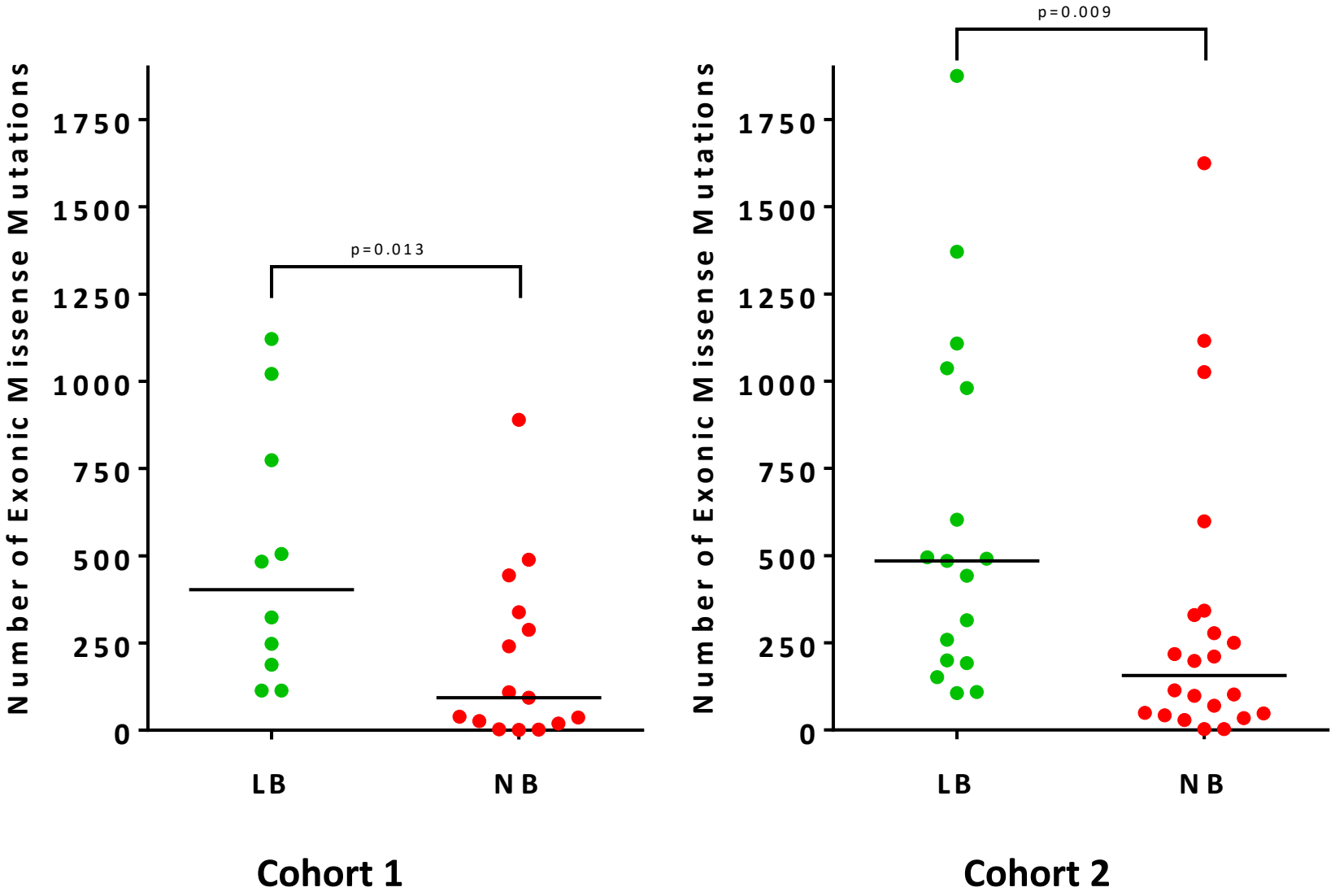
(Andersen AC et al Immunity 2016)



Immune Checkpoint Inhibitor Therapy More Effective when Blocking Multiple Check Point Molecular Pathways



Mutational Load Correlates with Clinical Outcome in Melanoma Patients Treated with α-CTLA-4



LB, long-term clinical benefit lasting ≥6 months
NB, no durable benefit

Snyder et al., *New Engl J Med*, 2014

FDA Grants Accelerated Approval to Pembrolizumab for First Tissue/Site Agnostic Indication Based on MMR Deficiency or MSI-H Status

- On May 23, 2017, the U.S. Food and Drug Administration granted accelerated approval to pembrolizumab (PD1) (KEYTRUDA, Merck & Co.) for adult and pediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options or with MSI-H or dMMR colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan.

A Sampling of Clinical Trials Combining Different Modalities with CP Inhibition to Increase Tumor Responses

Title	Recruitment	Study Results	Conditions	Interventions
A Study of Pembrolizumab And Platinum With Radiotherapy in Cervix Cancer	Not yet recruiting	No Results Available	• Cervix Cancer	• **Combination Product:** Pembrolizumab
Atezolizumab and Stereotactic Body Radiation Therapy in Treating Patients With Non-small Cell Lung Cancer	Recruiting	No Results Available	• Stage I Non Small Cell Lung Cancer	• **Drug:** Atezolizumab • **Radiation:** Stereotactic Body Radiation Therapy
Hypofractionated Radiation Therapy to Improve Immunotherapy Response in Non-Small Cell Lung Cancer	Recruiting	No Results Available	• Non Small Cell Lung Cancer Metastatic	• **Radiation:** Radiation • **Drug:** Immuno-Therapeutic Agent
Trial of SBRT With Concurrent Ipilimumab in Metastatic Melanoma	Completed	No Results Available	• Melanoma • Effects of Immunotherapy • Adverse Effect of Radiation Therapy	• **Radiation:** Stereotactic body • Radiotherapy (SBRT) • **Drug:** Ipilimumab
Phase I/II Study of the Anti-Programmed Death Ligand-1 Antibody MEDI4736 in Combination With Olaparib and/or Cediranib for Advanced Solid Tumors and Advanced or Recurrent Ovarian, Triple Negative Breast, Lung, Prostate and Colorectal Cancers	Recruiting	No Results Available	• Lung Cancer • Breast Cancer • Ovarian Cancer • Colorectal Cancer • Prostate Cancer • Triple Negative Breast Cancer	• **Drug:** Olaparib • **Drug:** Cediranib • **Drug:** MEDI4736

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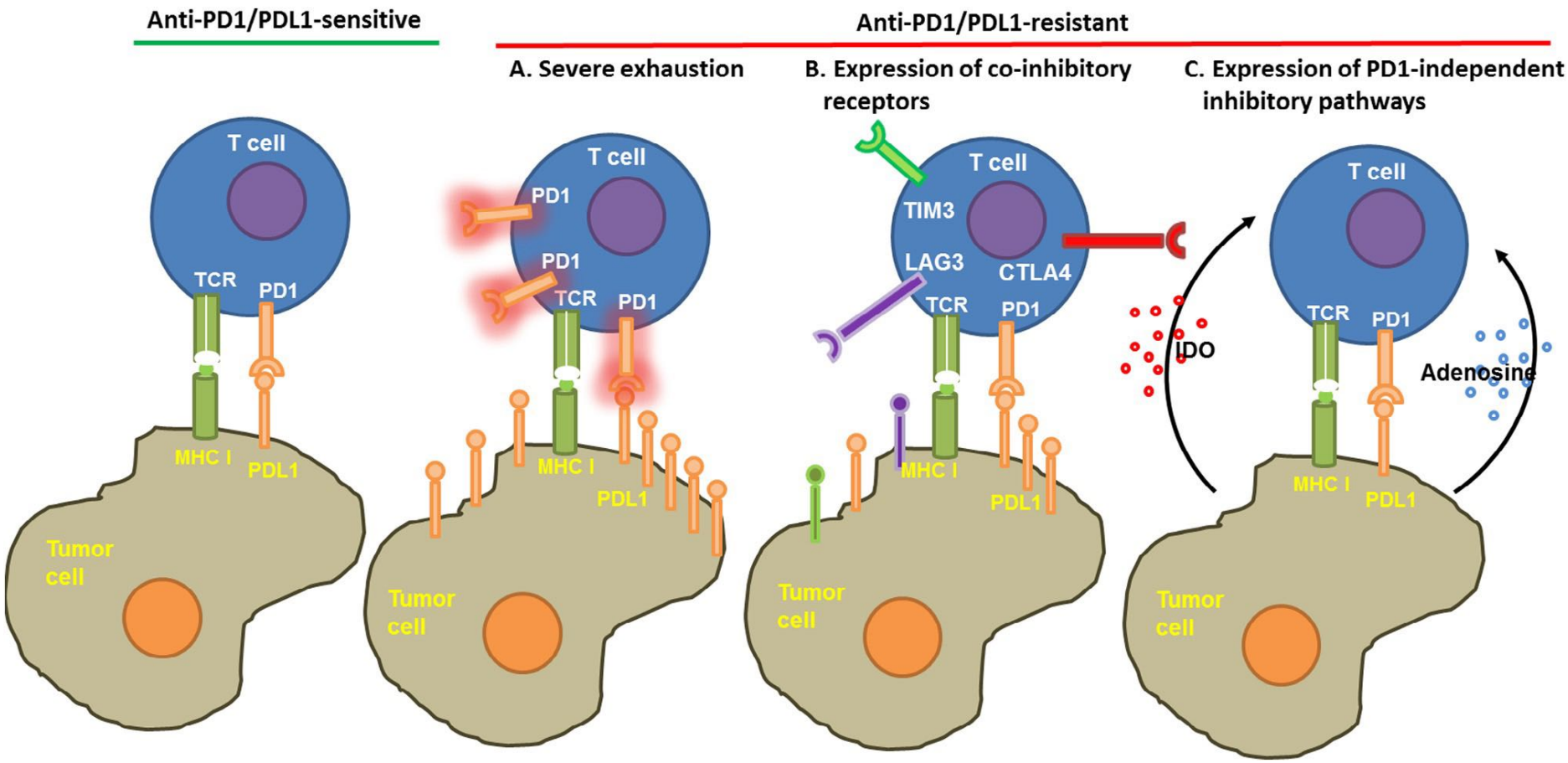
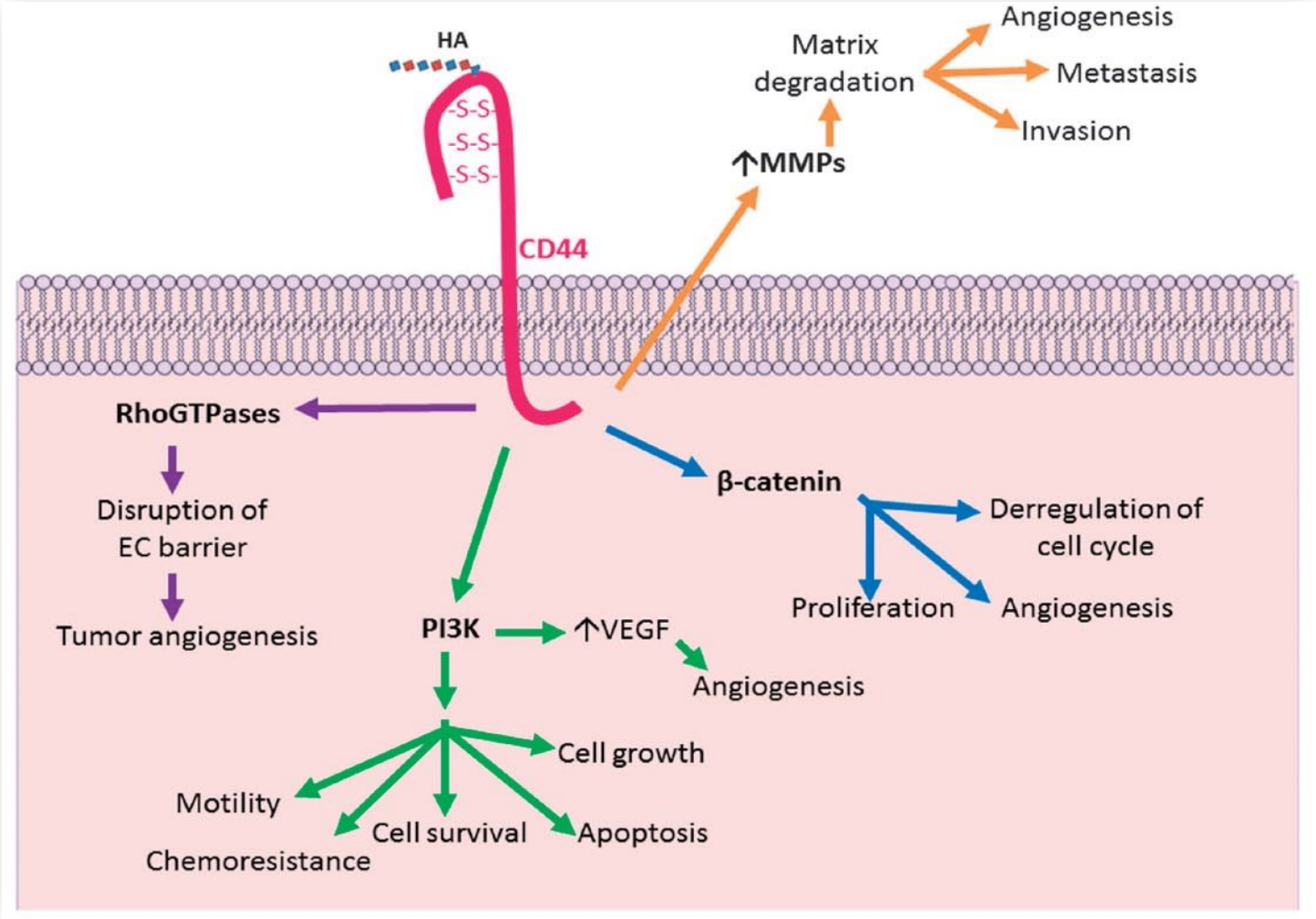
Mechanisms of Resistance to anti-PD1/PDL1 Therapies



O'Donnell JS et al Canc Treat Rev 2017

Remodeling of the Tumor Microenvironment to Facilitate Tumor Angiogenesis and Metastasis: Matrix Degradation Factors

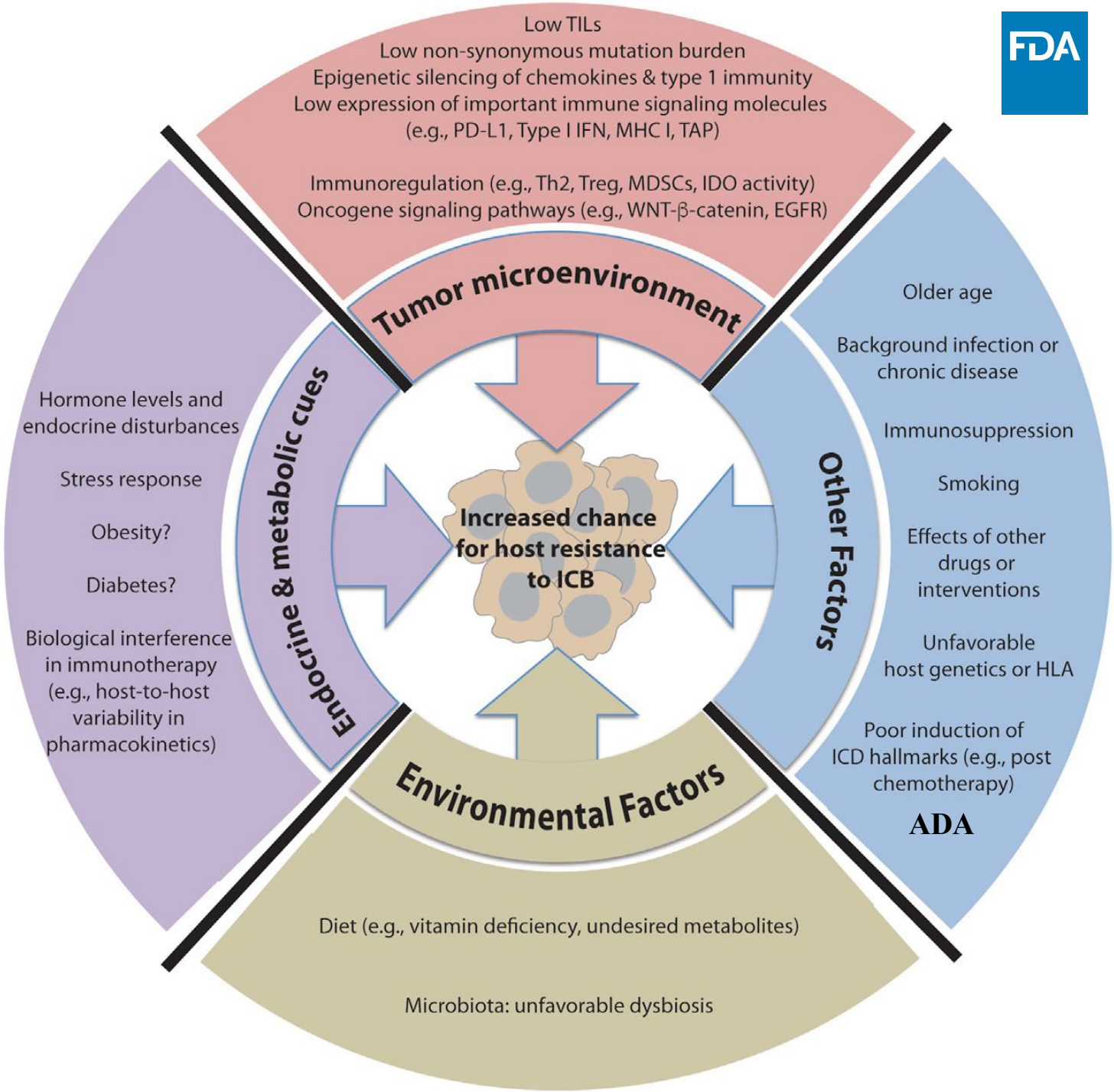


(Spinelli F et al 2015)

The Conundrum of Complete Responses: why is it ever possible to eliminate all tumor cells when a significant number are antigen loss variants?

- Bystander kill: non-antigen expressing tumors are killed directly via inflammatory mediators
- Destruction of microenvironment eliminates survival essentials for antigen loss tumors: structure and nutrient supply;
  - relies on cross presentation of tumor antigen by tumor stromal cells; ***requires high levels of neoantigen expression on tumors*** (Spiotto M et al Nat Med 2004)
- Epitope Spread: cross presentation elicits CTL to subdominant epitopes and to additional tumor (and potentially normal cellular) antigens
- Basis for abscopal effect? Activation of T cells to dominant or additional (subdominant) tumor antigens mediated by RT destruction of tumor cells

Factors Contributing to Resistance to Immunotherapy in Advanced Cancer



Pitt JM Immunity 2016

Anti-Drug Antibodies (ADA) to Checkpoint Monoclonal Antibodies Have the Capacity to Cause Resistance to Treatment

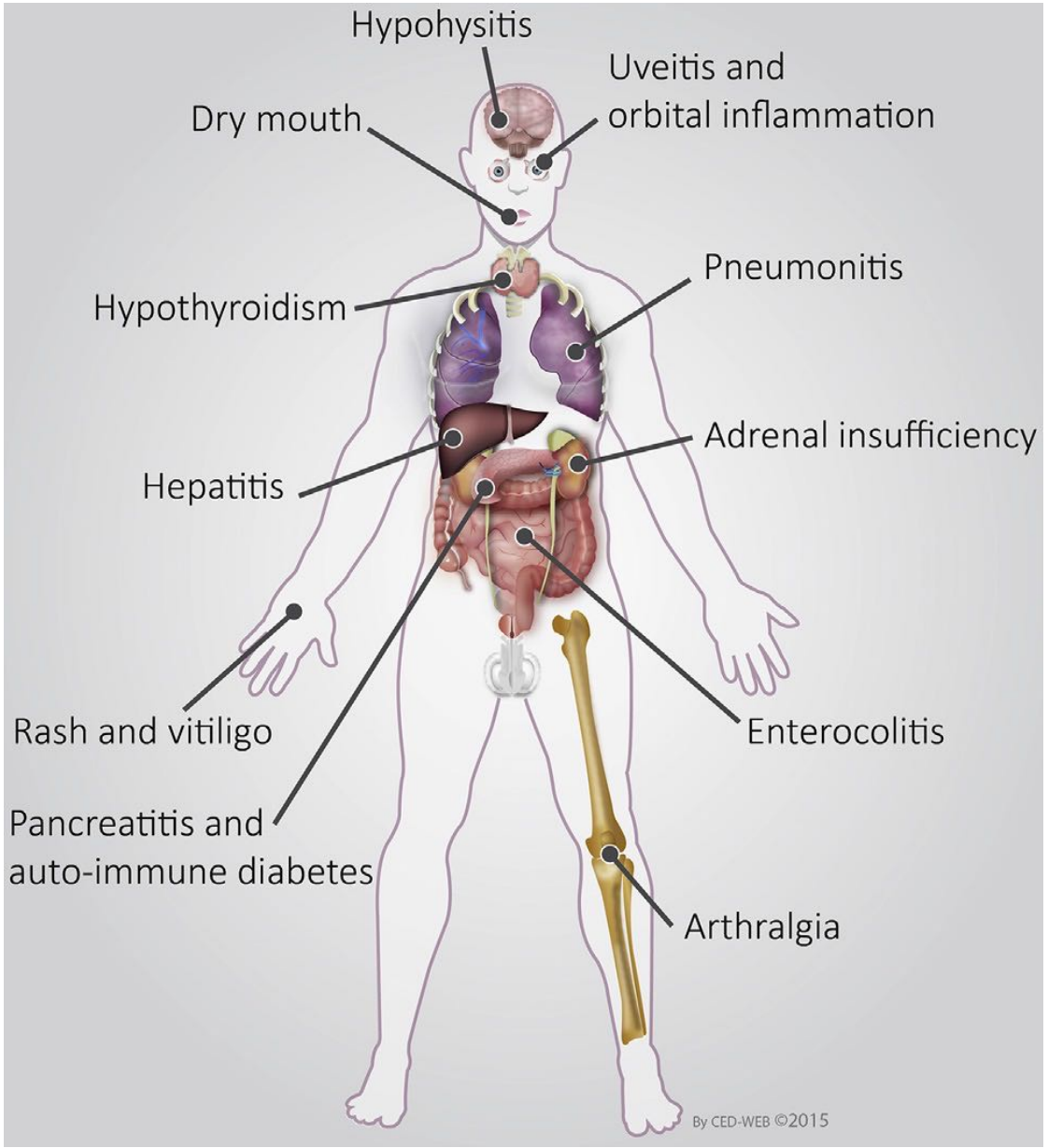
- Surprisingly low incidence of anti-drug antibodies to single agent check point monoclonal antibodies considering that immune inhibitory "brake is released": but higher when "***brakes"*** released
  - Pembrolizumab (anti-PD-1): 2% ADA;1 of 4 tested for NABs positive
  - Nivolumab (anti-PD-1): 11%; ***combined with Ipi-38%; ~5% NABs***
  - Ipilimumab (anti-CTLA4): 1.1%-4.9% ADA: ***combined with nivo -8.4%***
  - Avelumab (anti-PDL-1): 4.1%

- A function of immune dysfunction from numerous treatment courses of chemotherapeutic agents prior to immunotherapy?
  - Incidence of ADA to CP mAbs in 3rd/4th line treatment with immunotherapy vs incidence of ADA to CP mAbs as first line therapy?

Treatment of Advanced Cancer: Increasing Mutational Burden Boosting of Neoantigen and Activation of Immune Responses

- Novel combination strategies to increase efficacy
  - Increased mutational burden/neoantigen expression and the perpetuation of mutations is key to increasing immunogenicity of tumors:
    - immunogenic forms of tumor kill: RT, chemo, mutation targeted therapy, virologic/microbial therapy
    - factors that promote cross presentation and epitope spreading: importance of subdominant T cell clones
    - inhibitors of mismatch repair
    - addressing epigenetic modifications that silence neoantigen/MHC expression
  - Robust Activation of tumor specific T cells
    - Neoantigen vaccines
    - Agonist stimulation of T cells (eg OX-40)
    - CP blockade
    - Treg/MDSC Elimination
    - Inhibit suppressive factors (eg IDO), factors (MMP) that enhance tissue modifications for tumor spread/metastasis
- Intensification of treatment associated with expected and unexpected adverse events pertaining to autoimmunity: immune related adverse events

Always Potential Risk to Potential Benefit: The Clinical Spectrum of Immune-Related Adverse Events Associated with CP Inhibition



Michot JM et al 2016

Checkpoint Inhibitors Acting on Orthogonal Targets Have Greater Efficacy but also Greater Incidence and Severity of Immune Related AEs

(Larkin J et al NEJM 2015)

Event	Nivolumab (N=313)		Nivolumab plus Ipilimumab (N=313)		Ipilimumab (N=311)	
	Any	Grade 3 or 4	Any	Grade 3 or 4	Any	Grade 3 or 4
	number of patients with event (percent)					
Any adverse event	311 (99.4)	136 (43.5)	312 (99.7)	215 (68.7)	308 (99.0)	173 (55.6)
Treatment-related adverse event†	257 (82.1)	51 (16.3)	299 (95.5)	172 (55.0)	268 (86.2)	85 (27.3)
Diarrhea	60 (19.2)	7 (2.2)	138 (44.1)	29 (9.3)	103 (33.1)	19 (6.1)
Fatigue	107 (34.2)	4 (1.3)	110 (35.1)	13 (4.2)	87 (28.0)	3 (1.0)
Pruritus	59 (18.8)	0	104 (33.2)	6 (1.9)	110 (35.4)	1 (0.3)
Rash	81 (25.9)	2 (0.6)	126 (40.3)	15 (4.8)	102 (32.8)	6 (1.9)
Nausea	41 (13.1)	0	81 (25.9)	7 (2.2)	50 (16.1)	2 (0.6)
Pyrexia	18 (5.8)	0	58 (18.5)	2 (0.6)	21 (6.8)	1 (0.3)
Decreased appetite	34 (10.9)	0	56 (17.9)	4 (1.3)	39 (12.5)	1 (0.3)
Increase in alanine amino-transferase level	12 (3.8)	4 (1.3)	55 (17.6)	26 (8.3)	12 (3.9)	5 (1.6)
Vomiting	20 (6.4)	1 (0.3)	48 (15.3)	8 (2.6)	23 (7.4)	1 (0.3)
Increase in aspartate amino-transferase level	12 (3.8)	3 (1.0)	48 (15.3)	19 (6.1)	11 (3.5)	2 (0.6)
Hypothyroidism	27 (8.6)	0	47 (15.0)	1 (0.3)	13 (4.2)	0
Colitis	4 (1.3)	2 (0.6)	37 (11.8)	24 (7.7)	36 (11.6)	27 (8.7)
Arthralgia	24 (7.7)	0	33 (10.5)	1 (0.3)	19 (6.1)	0
Headache	23 (7.3)	0	32 (10.2)	1 (0.3)	24 (7.7)	1 (0.3)
Dyspnea	14 (4.5)	1 (0.3)	32 (10.2)	2 (0.6)	13 (4.2)	0
Treatment-related adverse event leading to discontinuation	24 (7.7)	16 (5.1)	114 (36.4)	92 (29.4)	46 (14.8)	41 (13.2)

* The safety population included all the patients who received at least one dose of study drug. The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

† The treatment-related adverse events listed here were those reported in at least 10% of the patients in any of the three study groups.

Parker Institute for Cancer Immunotherapy Autoimmunity and Cancer Consortium

Collaboration with multiple labs, researchers, universities, non-profits, government agencies, and pharma.

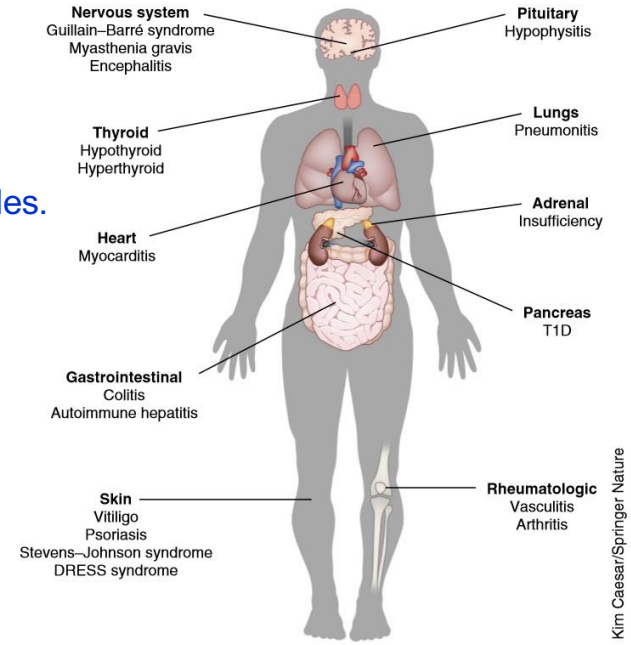
- **Goal**:
- Generate insight into the mechanisms behind immune-related adverse events (irAEs) following checkpoint inhibition in cancer patients.
- Early identification of at-risk patients to reduce the incidence and/or severity of such events.

Current:

- Initial focus on endocrinopathies.
- Four collaborative research projects being funded by PICI grants.
  Include pre-clinical models, clinical data mining, analyses of irAE patient samples.

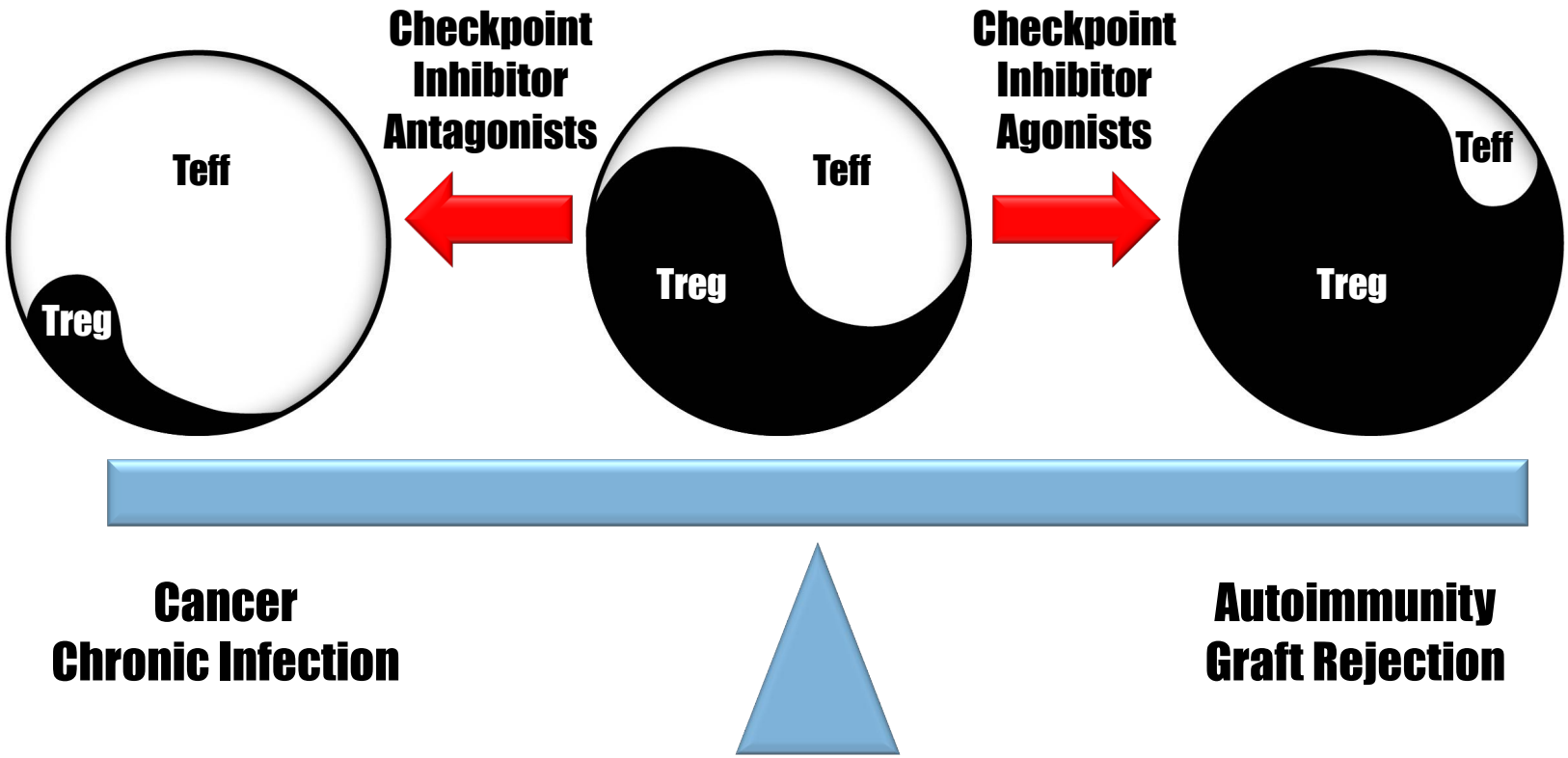
Moving forward:

- Collaborating with other non-profits to extend additional grants.
- Building biobank of patient specimens (pre- and post-treatment with CPIs) for additional research.
  - Prospective collections from patients receiving SOC.
  - Pharma collaboration - Banked clinical samples from patients who experienced irAEs.
- Pulling together a small group of Key Opinion Leaders from IO and autoimmunity.
  - Put together a plan for a more comprehensive project to study irAEs following CPI.



irAEs associated with cancer immunotherapy affect a wide range of organ systems

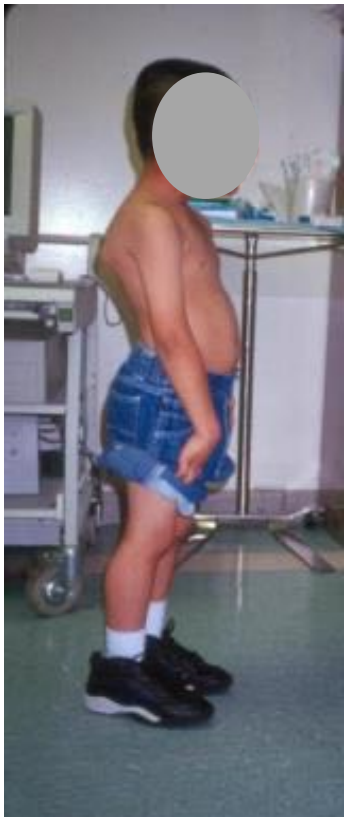
Manipulation of T-Cell Populations and Functions for Disease Indications

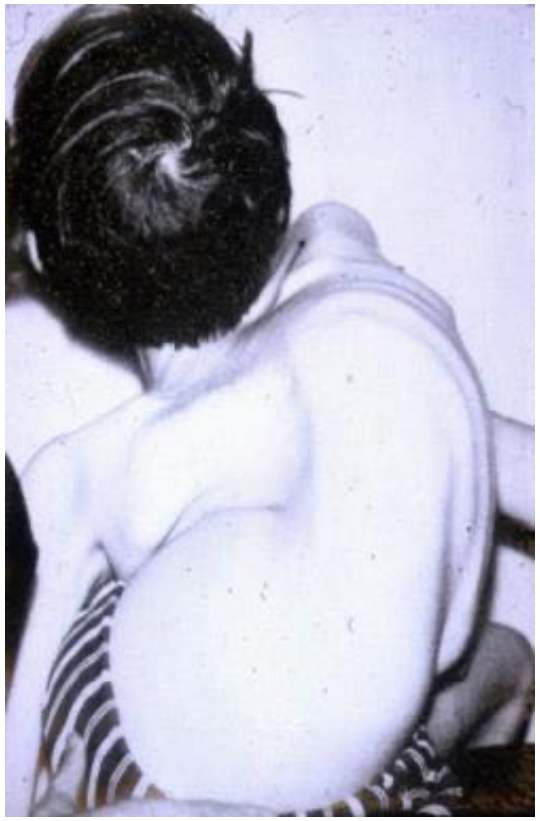


Duchenne muscular dystrophy (DMD)

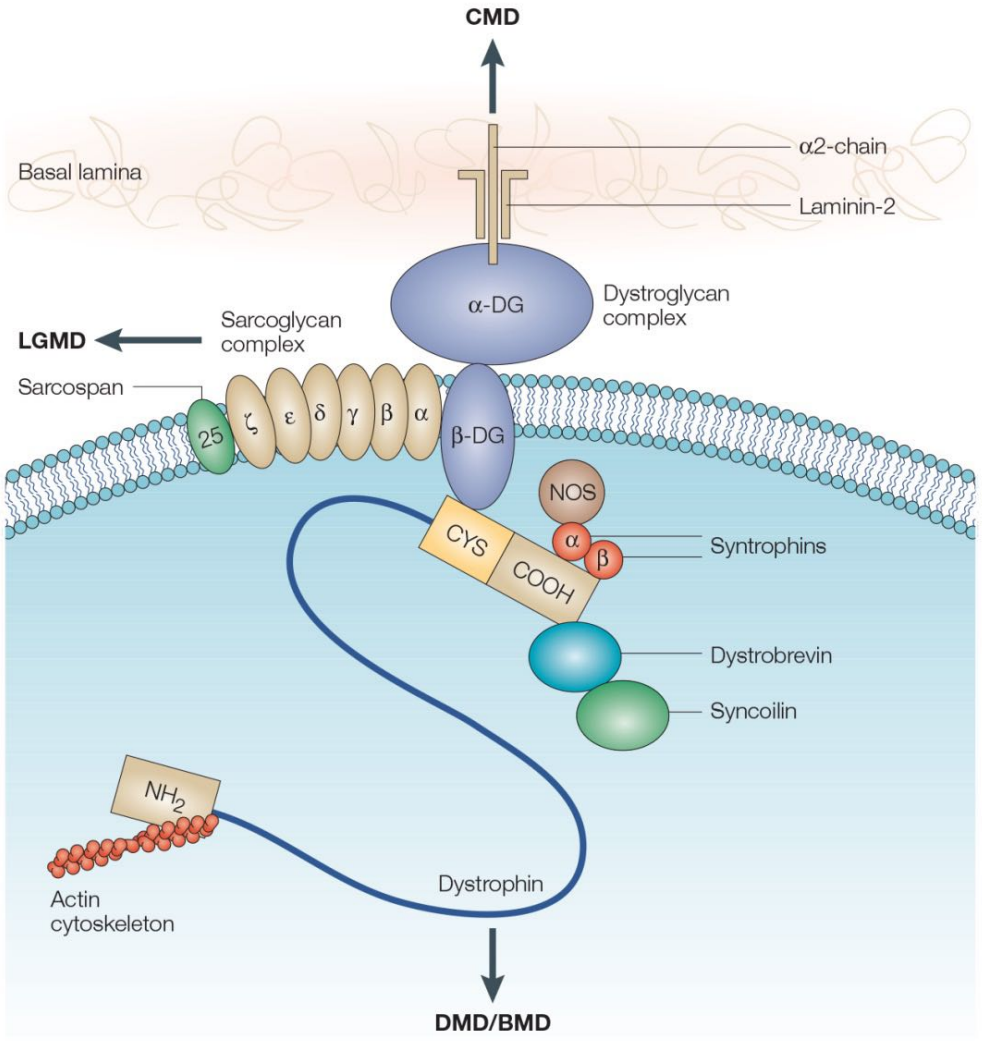
- DMD is an X-linked recessive, muscle-degenerative disorder.
- ***Most common inherited lethal disease of childhood***: ~ 1:3500 boys are affected.
- DMD is caused by mutations in the dystrophin gene, the majority of which result in the lack of functional protein.

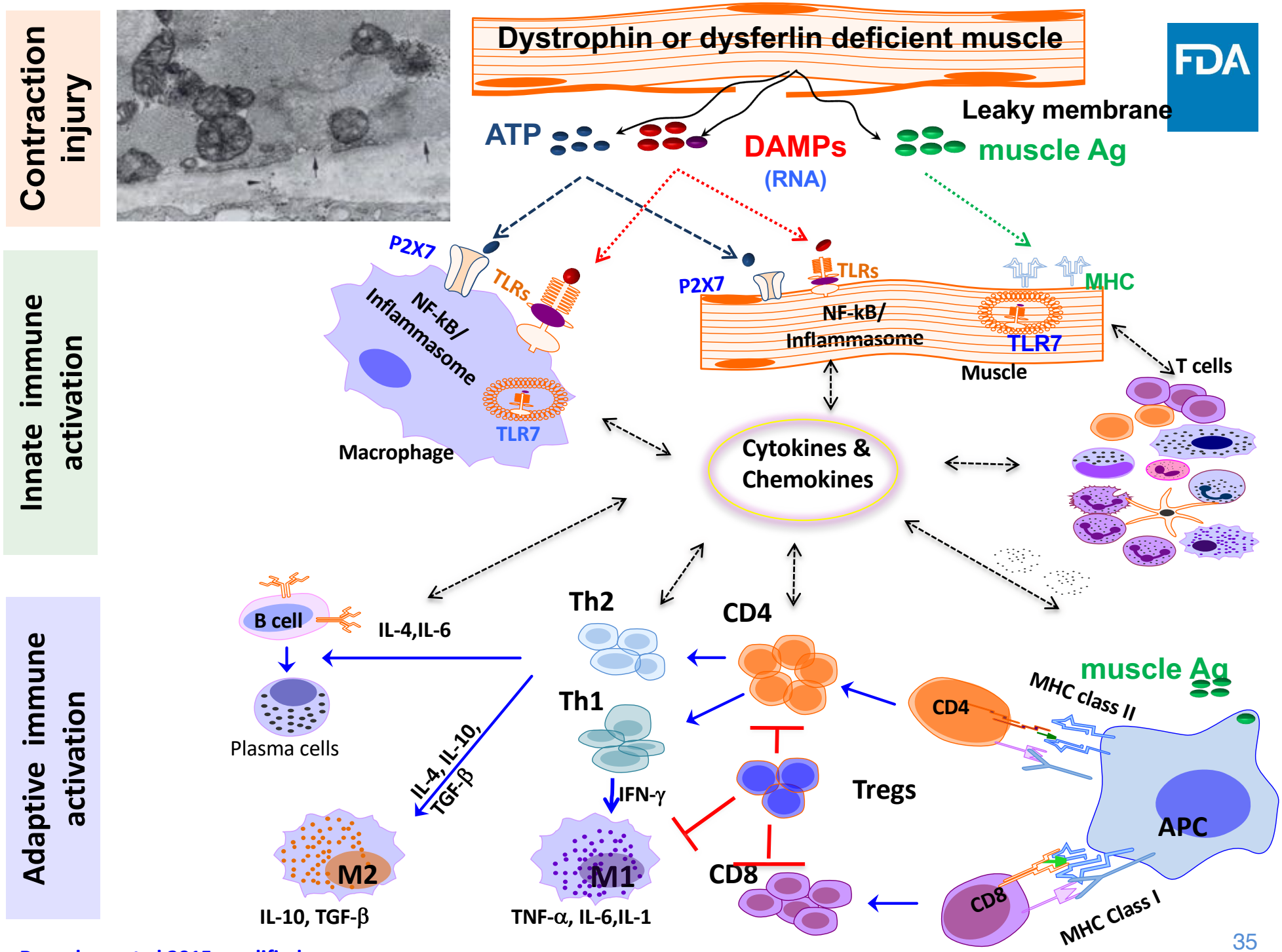
- Early decrease in muscle strength
- Loss of ambulation by adolescence
- Early death (early-mid twenties)





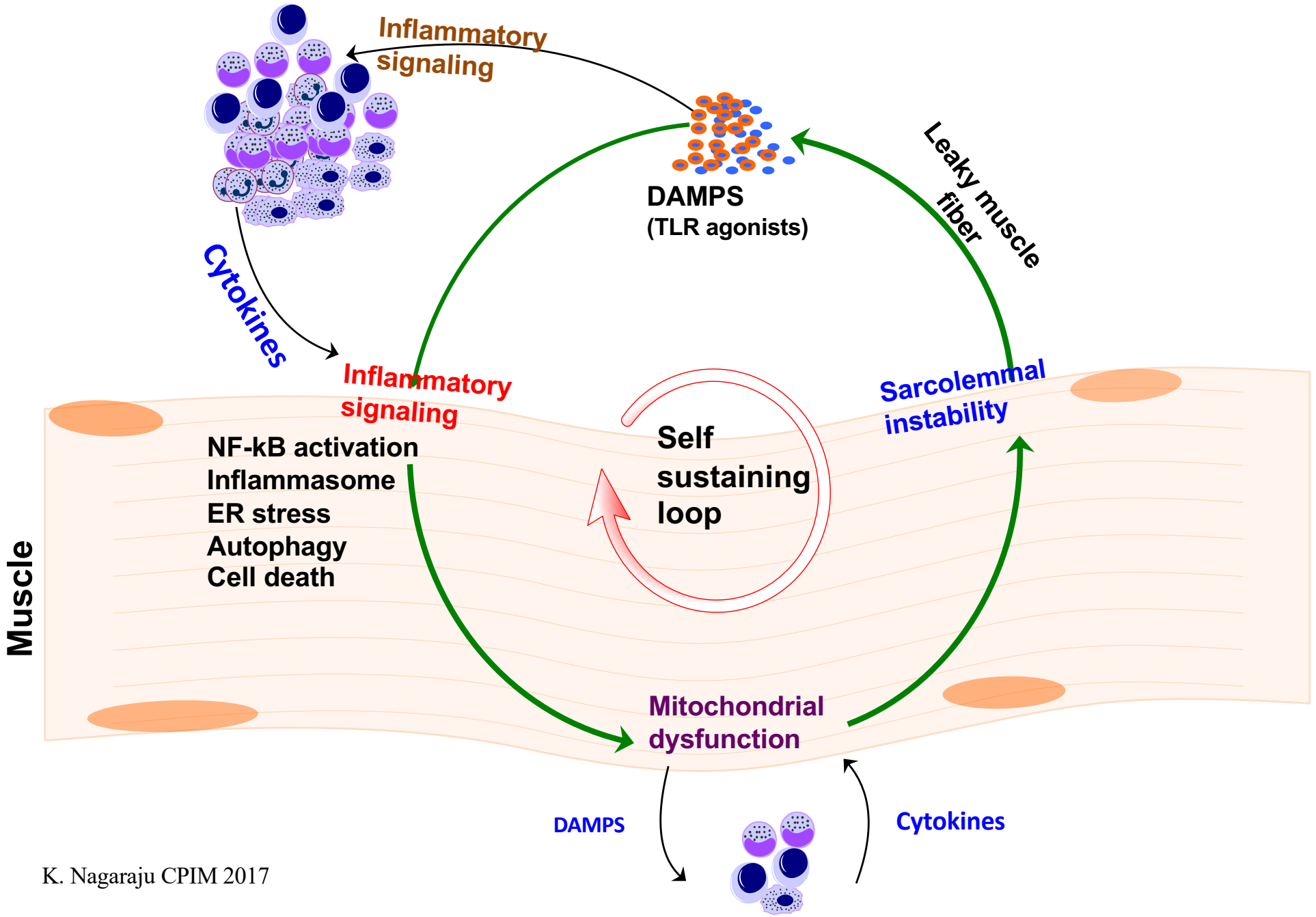
The Muscular Dystrophies and Organization of the Dystrophin–glycoprotein Complex





Rosenberg et al 2015-modified

Self Perpetuating Cycles of Inflammation in DMD



K. Nagaraju CPIM 2017

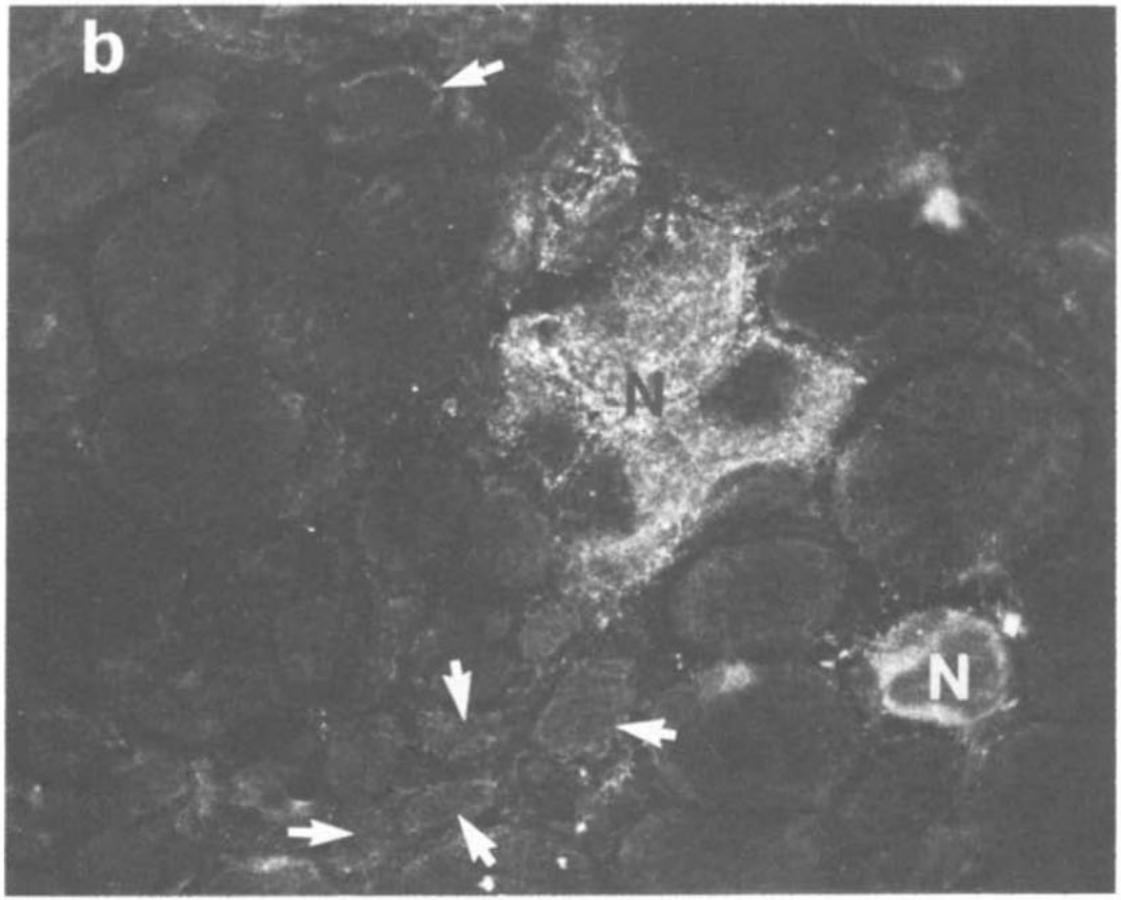
Additional Component of the Innate Immune Response: Upregulation of Complement Components and HLA class II in DMD

Affymetrix Accessions	Gene Description	Average Fold Changes	
		Infant	5–12 y
32527_at	cDNA clone IMAGE:2089315	2.9	4.5
36825_at	Staf50	1.7	3.2
36773_f_at	HLA-DQ-beta (DR7 DQw2)	1.9	3.1
41723_s_at	HLA-DR beta (DR2.3)	2.3	3.0
35822_at	Complement factor B	3.2	3.0
38095_i_at	HLA-DP beta	2.4	2.8
35016_at	Ia-associated invariant gamma-chain	2.0	2.8
38096_f_at	HLA-DP beta	2.9	2.7
38833_at	HLA-DPA1	2.0	2.7
37039_at	HLA-DR alpha	2.2	2.6
40282_s_at	Complement factor D	1.4	2.5
36878_f_at	HLA-DQ-beta (DQB1,DQw9)	2.4	2.4
35730_at	Alcohol dehydrogenase beta-1-subunit	2.9	1.7

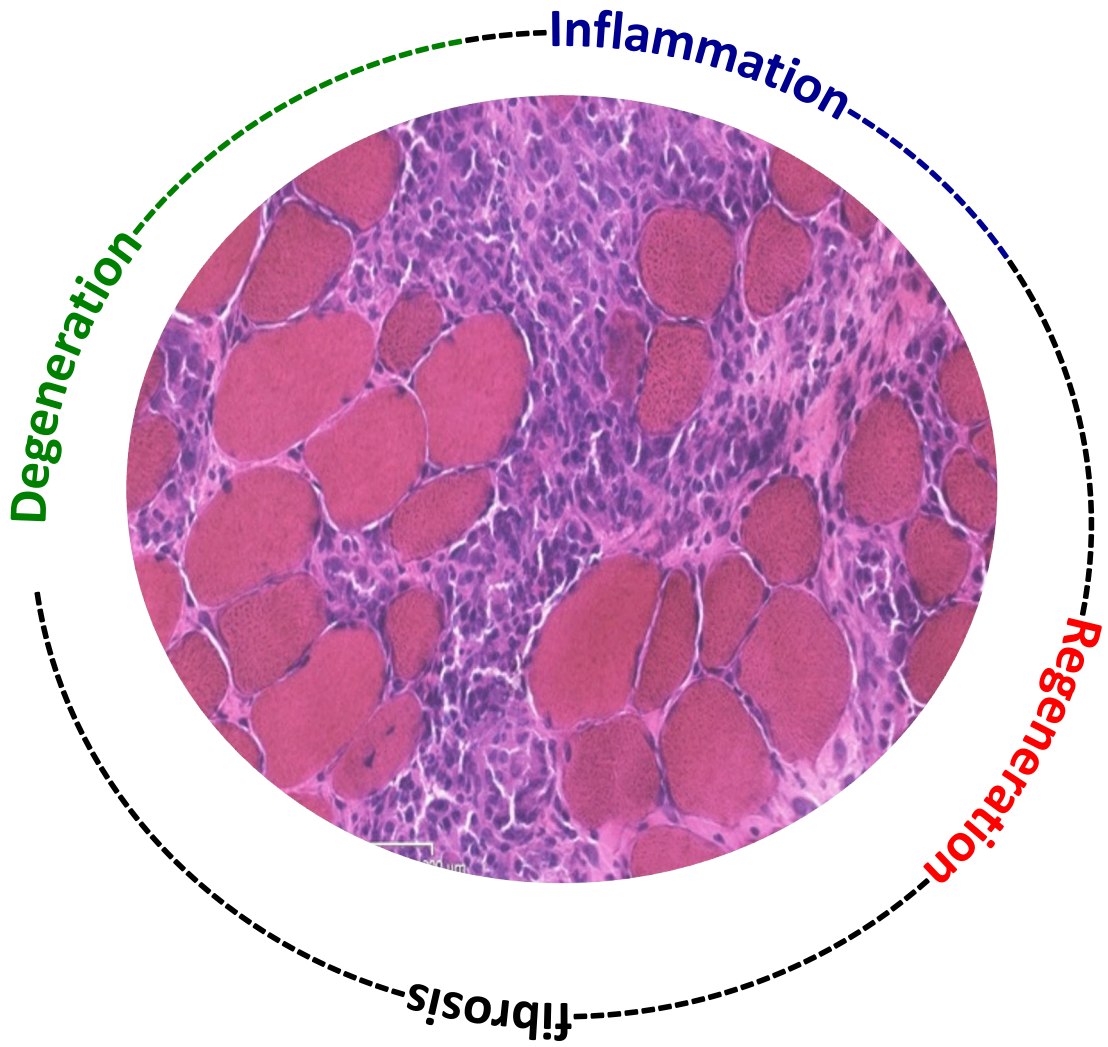
828 NEUROLOGY 65 **September (2 of 2) 2005**

Necrotic Fibers in DMD Label Intensely for Complement Membrane Attack Complex

(Sewry CA et al 1987)



Successive Cycles of Inflammation, Degeneration and Regeneration Terminate in Muscle Fibrosis, Loss of Function and Death



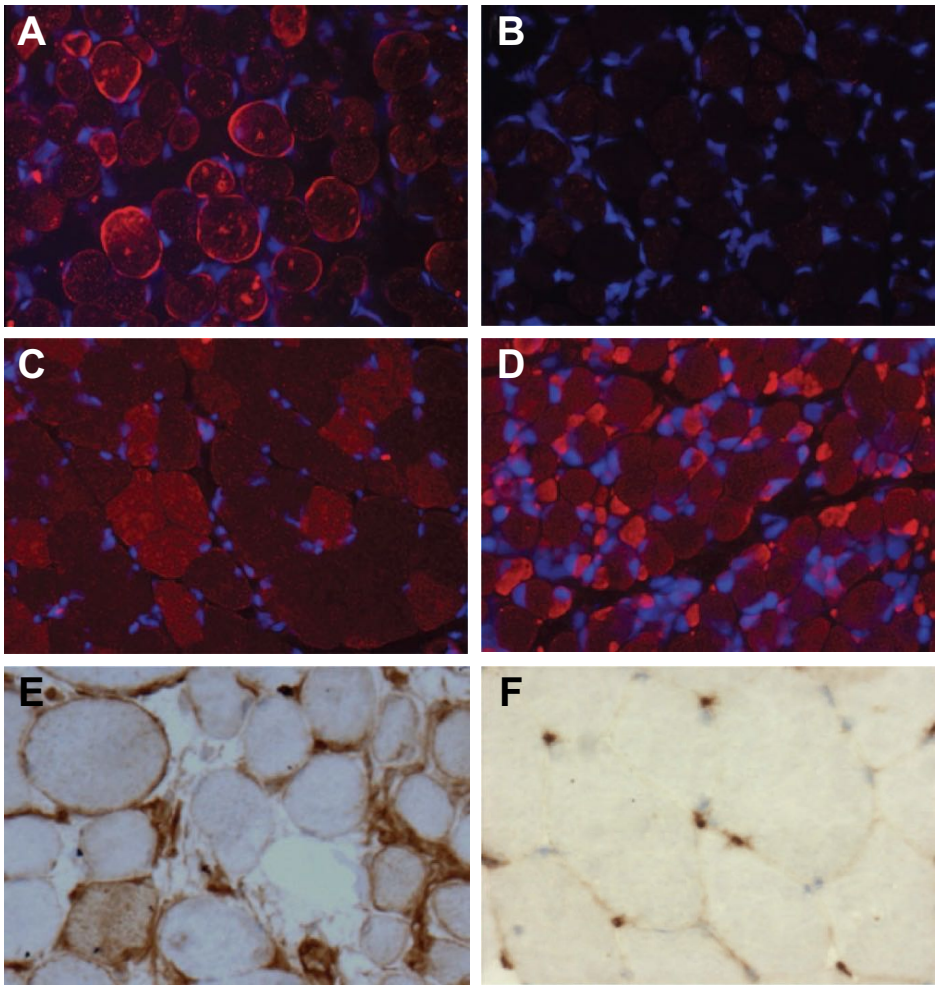
Adapted from K. Nagaraju CPIM 1/17

Bridge Between Innate and Adaptive Immunity: NF-κB Activation by TLR Signaling Induces HLA Class I Expression on Dystrophic Muscle and Antigen Presentation

p65 DMD

p65 Cont

HLA Class I DMD



Dystro Def Muscle

HLA Class I Cont

Chen YW et al Neurology 2005

Strategies to Diminish Inflammatory Response-Fibrosis Cycle in DMD

- Newborn screening for early diagnosis: inflammation begins long before clinical diagnosis; dystrophin immunity correlates with age/negatively correlates with steroid treatment

- Diminish the innate immune response: use as window for dystrophin tolerance induction
  - Anti-inflammatory therapies:
    - Steroids: currently the standard of care; diminishes dystrophin responses and offers a window for more effective long term therapies but significant toxicities.
    - TLR and/or NFkB antagonists;
    - Complement attack complex inhibitors: ecalizumab
    - Inflammatory cytokine antagonists: a-TNF, IL-1?
    - IVIG for immune modulation and protection against infection

Critical Path Innovation Meeting on DMD
January, 2017

- **Key Question**: Dr. Woodcock asked whether the experts present thought that it would be necessary to replace dystrophin if it was possible to shut down inflammation in DMD:
  - Consensus was that it was necessary to replace dystrophin: "Even if repeated rounds of regeneration were possible, it would likely exhaust the cell lines"
  - "Targeting inflammation is a very worthy goal, but it still doesn't get to the problem of dystrophin deficiency. .. we have to think of using that window (shutting down inflammation) to decrease inflammation and increase regulatory T cells and tolerance to dystrophin to get a better, more definitive therapy"

DMD is a Monogenic Disease: Dystrophin Replacement Therapy Should be Effective but….

- Preexisting, age correlated, dystrophin immunity in a substantial percentage of patients: revertant fiber expression of dystrophin at low levels *and in an inflammatory environment that promotes HLA expression on muscle* primes dystrophin responses rather than tolerizes: dystrophin vaccine
- In DMD patients not already immunologically primed to dystrophin, it appears as a neoantigen as the majority have frame shift mutations leading to nonsense mediated decay of dystrophin mRNAs and lack of protein expression;
- Dystrophin gene therapy elicits primary or boosts memory dystrophin specific immune responses
- Patients may have preexisting immunity to AAV vector further diminishing gene transduction and expression: cutoff of 1:50 antibody titer to AAV suggested for clinical trials. Development of less immunogenic vectors and approaches critical.

Oligoclonal T Cell Populations at the Site of Muscle Degeneration in Duchenne Muscular Dystrophy: Specificity for Dystrophin?

DMD Patients	Vβ1	Vβ2	Vβ3	Vβ4	Vβ5.1	Vβ5.2	Vβ6	Vβ7	Vβ8	Vβ9	Vβ10	Vβ11	Vβ12	Vβ13	Vβ14	Vβ15	Vβ16	Vβ17	Vβ18	Vβ19	Vβ20
84	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
85	–	+	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
86	+	+	+	–	–	+	+	+	–	–	–	–	–	–	+	–	–	–	–	–	–
5																					
pre-implant	–	+	+	–	–	+	–	+	–	–	–	–	–	+	–	–	+	–	–	–	–
1 mo post	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6 mo post	–	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6																					
pre-implant	–	+	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
1 mo post	–	+	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
6 mo post	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8																					
pre-implant	–	+	–	–	–	–	–	+	+	–	–	–	–	+	–	–	–	–	–	–	–
1 mo post	+	+	+	–	–	–	–	+	+	–	–	–	–	–	+	–	–	–	–	–	–
6 mo post	+	+	+	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–

[a]Quantitation of TCR transcripts was accomplished through amplification of a Cα-Cα region as an internal control for variation among samples (28), con-comitantly with a TCR β-chain transcript, to assess the relative amount of each of 20 known TCR Vβ families. Quantity of TCR Vβ transcripts was expressed as a percentage of the quanity of the co-amplified Cα-Cα transcripts. In this table, the symbol + indicates values greater than 5%, and – indicates values <5%.

Gussoni E et al J. Immunol 1994

Selection for the RVSG Motif in TCR of T cells Infiltrating Muscle in DMD

Patient #5

Pre-implant

TGC	AGT	GCC	CAG	CGT	GTG	TCT	GGA	AAC	
C	S	A	Q	R	V	S	G	N	Jβ 1.3 (Ψ)

Myoblast-injected leg 1 mo after transplant

TGC	AGT	GCC	CAG	CGT	GTG	TCT	GGA	AAC	
C	S	A	Q	R	V	S	G	N	Jβ 1.3

Placebo-injected leg 1 mo after transplant

TGC	AGT	GCC	CAG	CGT	GTG	TCT	GGA	AAC	
C	S	A	Q	R	V	S	G	N	Jβ 1.3 (Ψ)
TGC	AGT	GCA	GGG	AGG	GTC	TCT	GGA	AAC	
C	S	A	G	R	V	S	G	N	Jβ 1.3
TGC	AGT	GCT	AGG	AGG	GTG	TCT	GGA	AAC	
C	S	A	R	R	V	S	G	N	Jβ 1.3

Myoblast-injected leg 6 mo after transplant

TGC	AGT	GCT	AGC	CGA	GTA	TCT	GGA	AAC	
C	S	A	S	R	V	S	G	N	Jβ 1.3

Patient #6

Pre-implant

TGC	AGT	GCT	TCT	CGG	GTC	TCT	GGA	AAC	
C	S	A	S	R	V	S	G	N	Jβ 1.3 (Ψ)

Placebo-injected leg 1 mo after transplant

TGC	AGT	GCT	TCT	CGG	GTC	TCT	GGA	AAC	
C	S	A	S	R	V	S	G	N	Jβ 1.3

Myoblast-injected leg 6 mo after transplant

TGC	AGT	GCT	AAC	AGG	GTC	TCT	GGA	ACA	
C	S	A	N	R	V	S	G	N	Jβ 1.3

Patient #8

Pre-implant

TGC	AGT	GCT	AGT	AGG	GTG	TCC	GGT	GAA	
C	S	A	S	R	V	S	G	E	Jβ 1.4

Placebo-injected leg 1 mo after transplant

TGC	AGT	GCT	CAG	AGG	GTG	TCG	GGA	ACA	
C	S	A	Q	R	V	S	G	T	Jβ 1.4

Myoblast-injected leg 6 mo after transplant

TGC	AGT	GCT	CAG	AGG	GTG	TCG	GGA	ACA	
C	S	A	Q	R	V	S	G	T	Jβ 1.4

Patient #4

Placebo-injected *leg 1 mo after transplant*

TGC	AGT	GCC	TTG	AGG	GTG	TCG	GGC	ATT	
C	S	A	L	R	V	S	G	N	Jβ 2.1 (Ψ)

Patient #86

TGC	AGT	GCT	AGT	AGG	GTT	TCT	GGA	AAC	
C	S	A	S	R	V	S	G	N	Jβ 1.3 (¶)

[a]Nucleotide and amino acid sequences of the Vβ2 T cells expressing the RVSG CDR3 motif. In some samples, clones with an identical nucleotide sequence were found more than one time. The symbol (Ψ) designates clones found two times, and (¶) designates clones found five times in the same sample

Gussoni E et al J. Immunol 1994

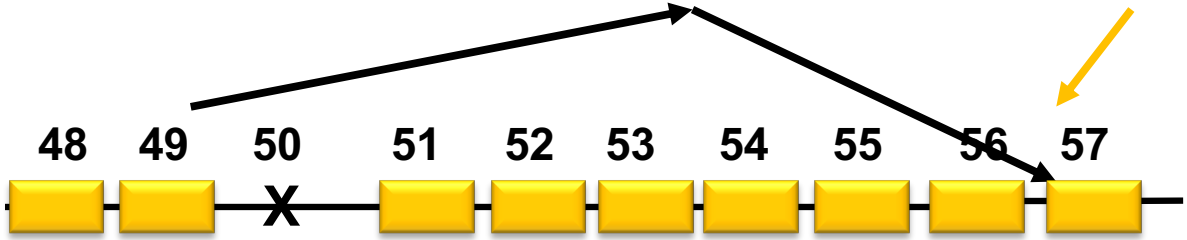
Evidence of Preexisting Immunity to Dystrophin: Revertant Fiber Expression Priming

Patient ID	Treatment Regimen	Mutation[a]	Truncating Mutation (prediction)	Location of Immune Response	Location of Response Relative to Mutation Location	T Cell Phenotype
12	Naïve	Splice exon 12	+	Exons 42-50	Downstream	CD4
21	Prednisone	Del ex 45	+	Exons 1-9	Upstream	CD4
74	Naïve	Del ex 46-50	+	Exons 42-50	Upstream	CD8
39	Naïve	Del ex 48-50	+	Exons 42-50	Upstream	CD4
19	Naïve	Del ex 48-50	+	Exons 17-26	Upstream	CD4/CD8
35	Prednisone	Del ex 50	+	Exons 50-59	Downstream	CD4
11	Naïve	Del ex 49-54	+	Exons 17-26	Upstream	CD8
14	Naïve	Nonsense ex 59	+	Exons 70-79	Downstream	CD4
59	Prednisone	Nonsense ex 69	+	Exons 59-69	Uostream	CD4

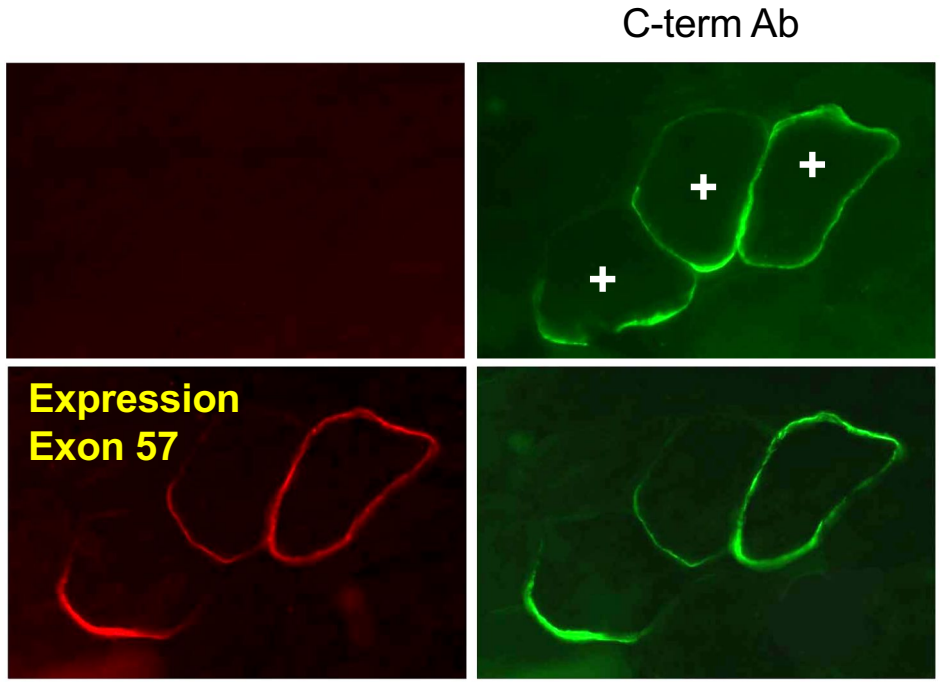
[a]Del, deletion; ex, exon. All are truncating mutations that are predicted to result in an interrupted mRNA reading frame.

Flanigan KM et al HUMAN GENE THERAPY 24:797–806 2013

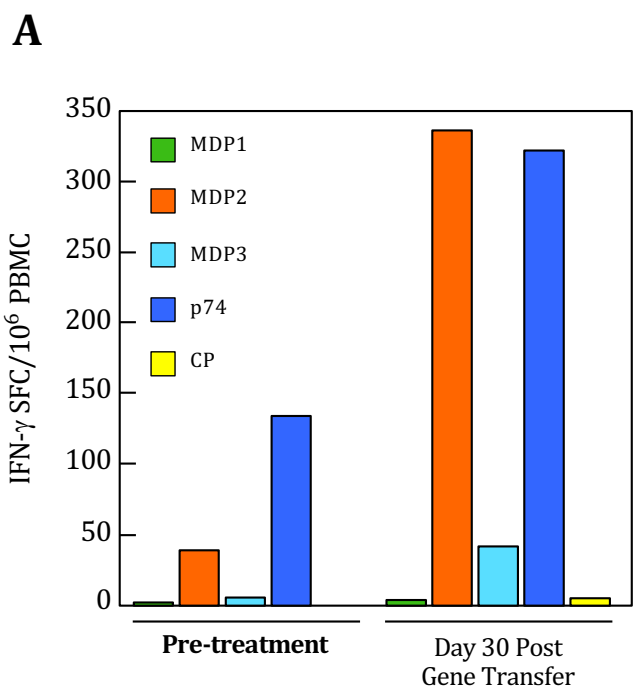
Pre-existing Immunity to Misfolded Protein Expressed on Revertant Fibers

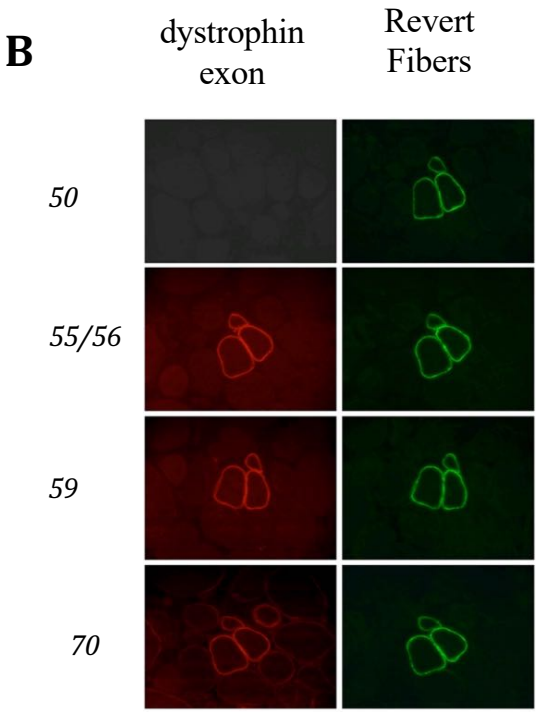


- Patient Deleted for Exon 50
  - Frameshift mutation
  - +Revertant Fibers
    - Second site mutation restores reading frame "Spontaneous exon skipping to exon 57"



Dystrophin Immunity Present Prior to and Boosted by Gene Therapy with mini-Dystrophin Cassette in Patient with Exon 50 Deletion





MDP1: Exons 1-11/12
MDP2: Exons 12, 50-51, 56-59
MDP3: Exons 59-70
p74: Exon 57;aa 2809-2829

Mendell JR et al NEJM 2010

Strategies to Preclude Dystrophin Immunity Require Administration of Replacement Therapy in Optimized Setting

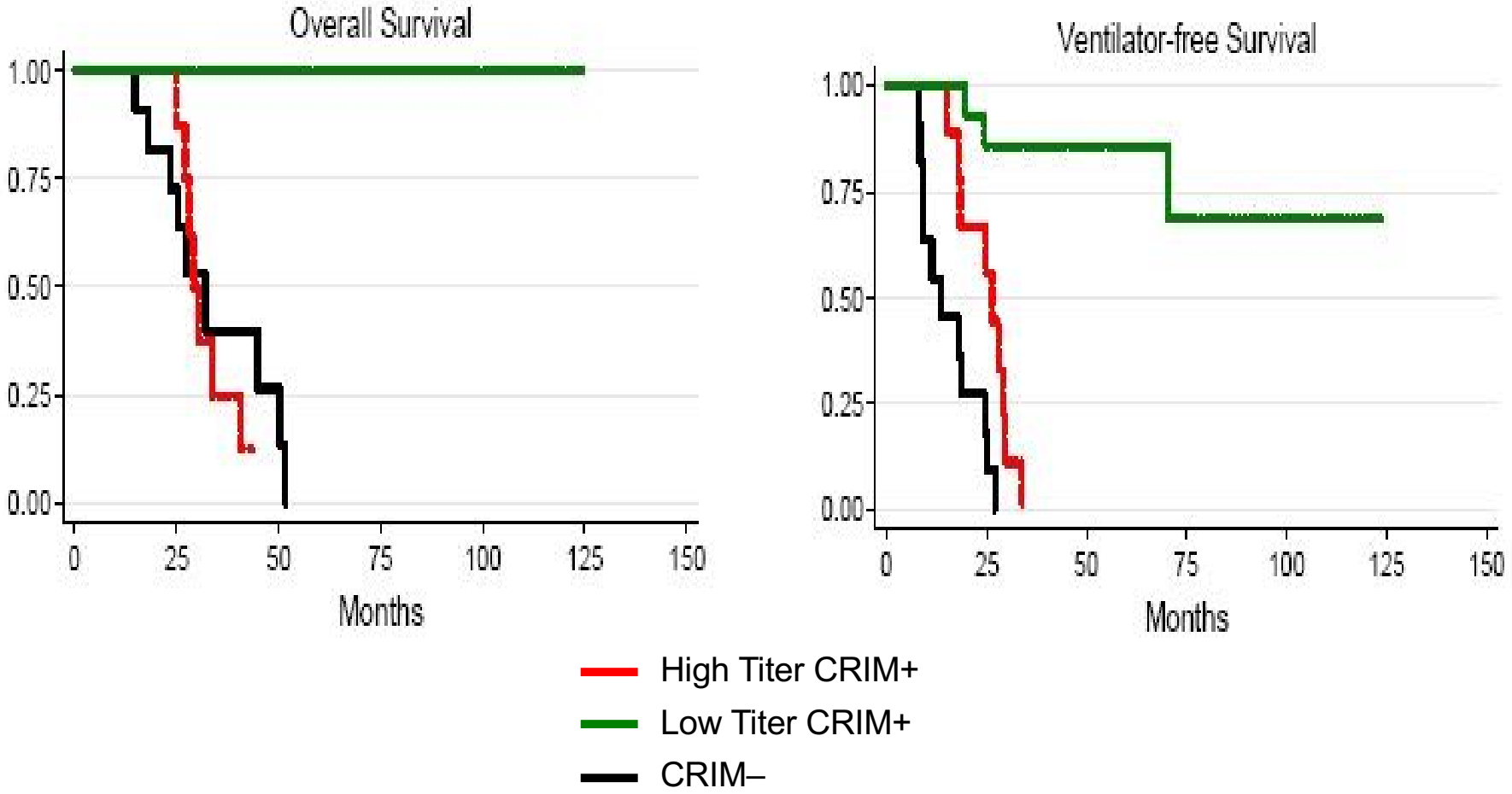
- Newborn screening for early diagnosis: dystrophin immunity correlates with age and negatively correlates with steroid treatment
- Diminish the innate immune response
  - Anti-inflammatory therapies:
    - Steroids: currently the standard of care; diminishes dystrophin responses and offers a window for more effective long term therapies.
    - TLR and/or NFκB antagonists;
    - Complement attack complex inhibitors: ecalizumab
    - Inflammatory cytokine antagonists: a-TNF, IL-1?
    - IVIG for immune modulation and protection against infection
- Preclude or Eliminate Dystrophin Specific Immunity: Immune Tolerance Induction
  - Tolerance induction protocol for cell mediated (eg CD8+ T cells) immune responses
  - Tolerance induction in the context of transplantation and gene therapy appropriate disease models

Mitigation of Immune Responses to Life Saving Therapeutics

- When consequences of immune responses to biological therapeutics 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

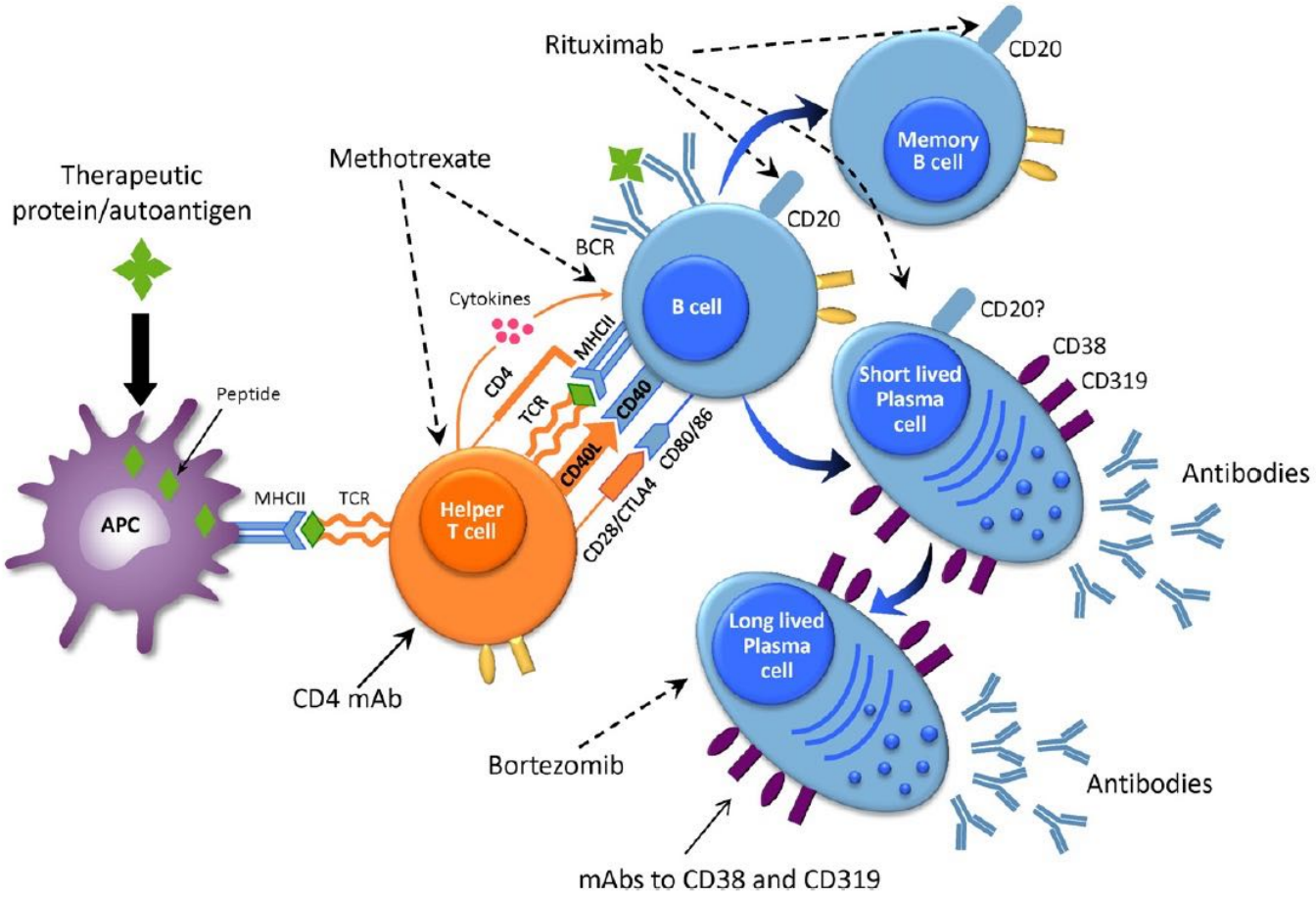
- Protein engineering to "deimmunize" a protein therapeutic and development of mimetics that lack amino acid or epitope homology
    - Use of predictive algorithms and in vitro studies to identify and remove immunogenic epitopes
    - protein engineering should ensure that other critical attributes of the therapeutic protein are not altered for the worse such as enhanced aggregation, oxidation, deamidation etc

Requirement for Immune Tolerance Induction in Pompe Disease: Robust Immune Response Neutralizes Life Saving Enzyme Replacement Therapy in Pompe Disease

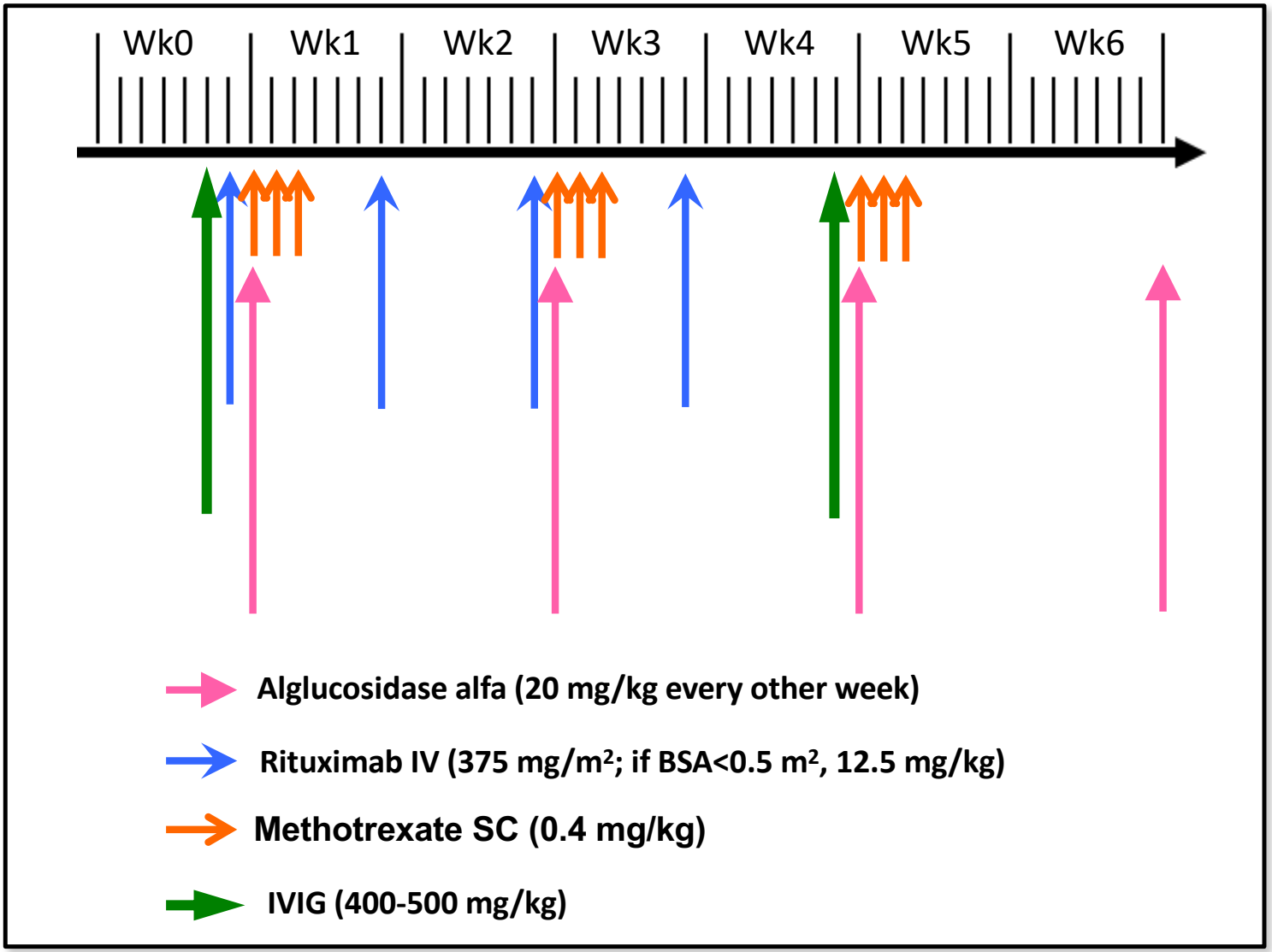


(Kishnani PS et al 2011)

Rituximab to Target B Cells and Methotrexate to Target Antigen Activated T and B Cells for Prophylactic Immune Tolerance to ERT



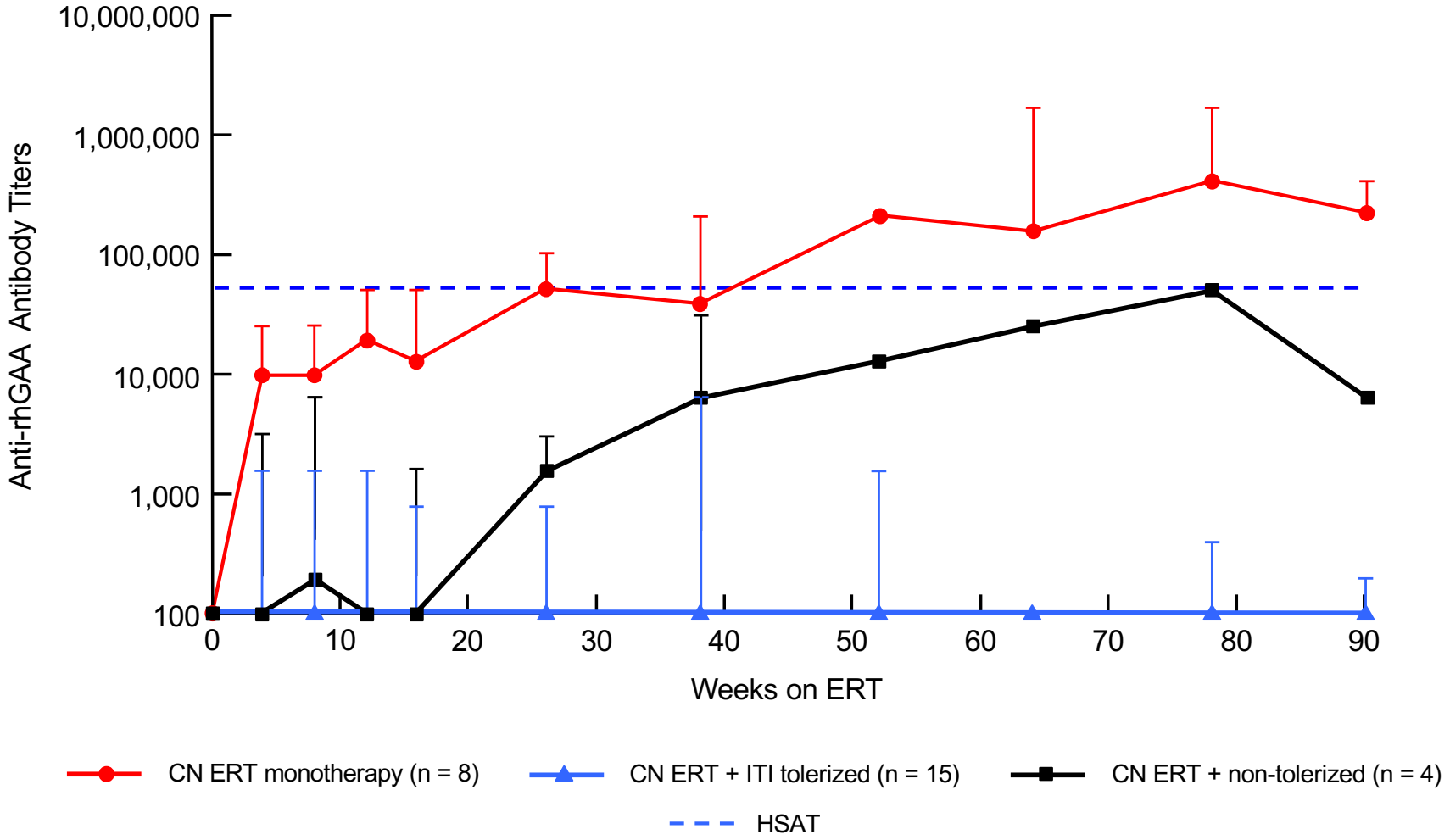
Prophylactic ITI Protocol



Banugaria S et al PlosOne 2013

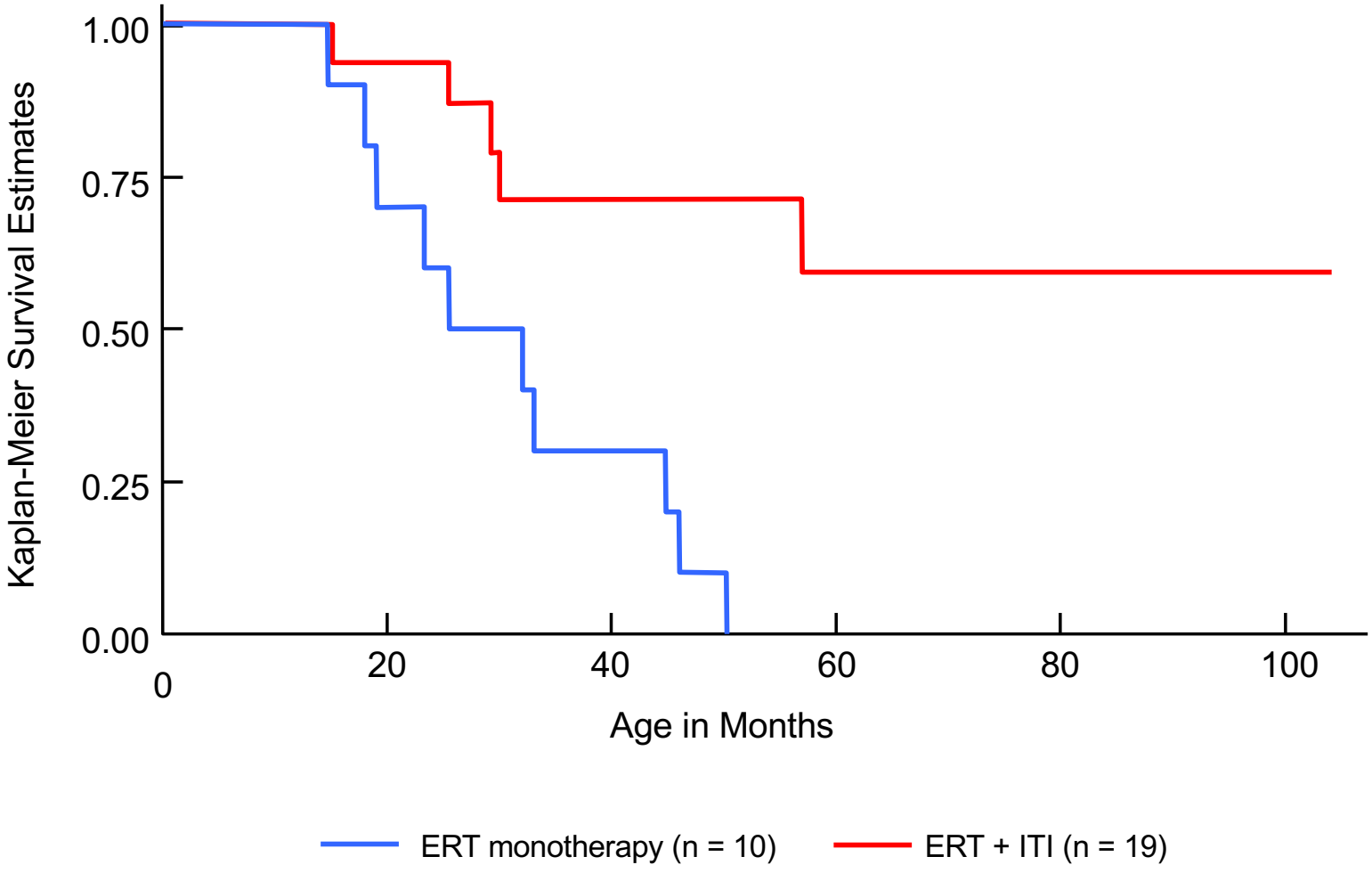
rhGAA Antibody Titer in CRIM-negative IPD Patients Treated Prophylactically with ERT+ITI versus ERT Monotherapy

(Kazi ZB et al JCI Insight 2017)



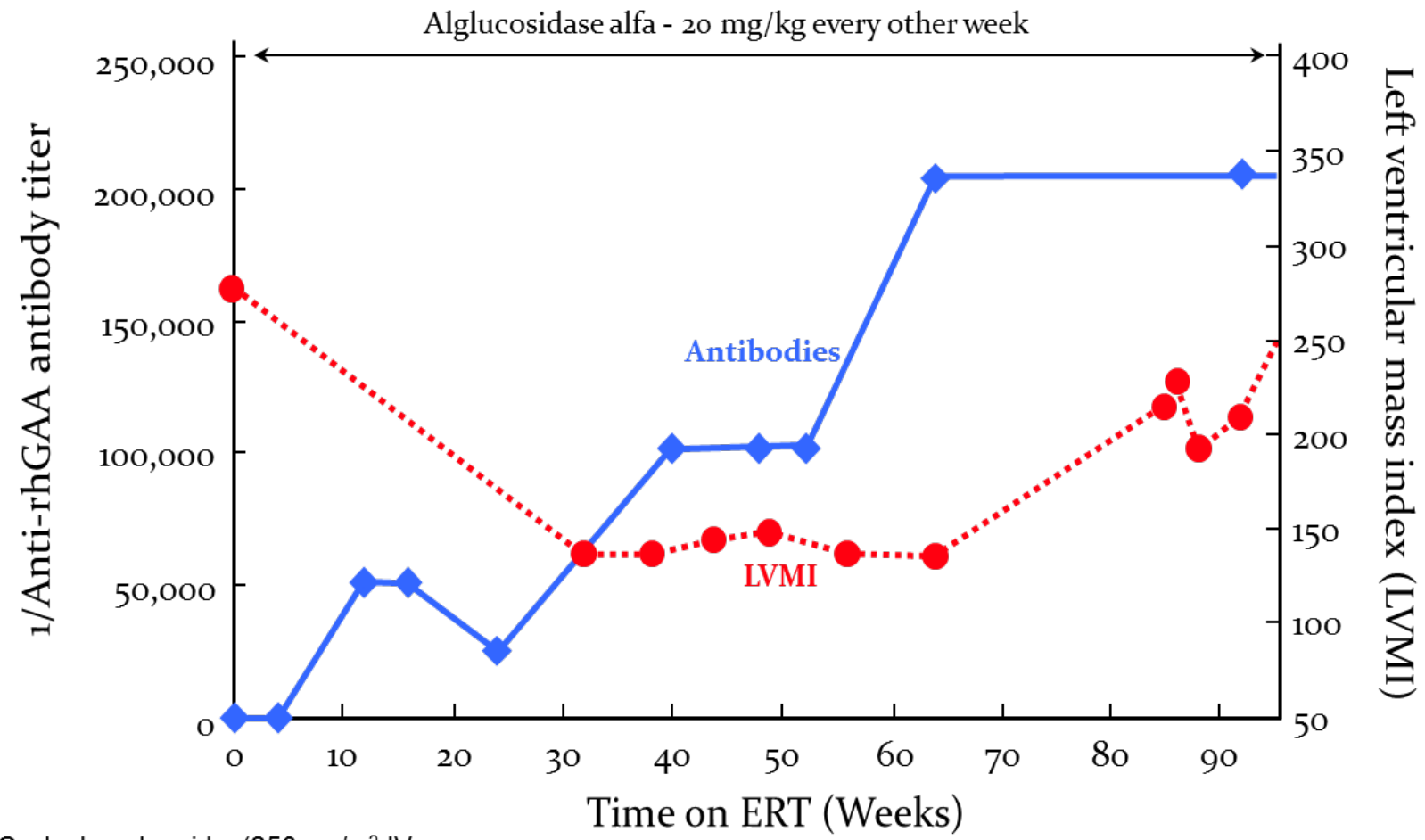
Survival of CRIM- IPD Patients Treated Prophylactically with ERT+ITI versus ERT Monotherapy

(Kazi ZB et al JCI Insight 2017)



High Titer and Sustained Antibody Responses: Unresponsive to Immune Suppressive Agents used in Prophylactic Regimen

(Banugaria SG et al Genet Med 2013)



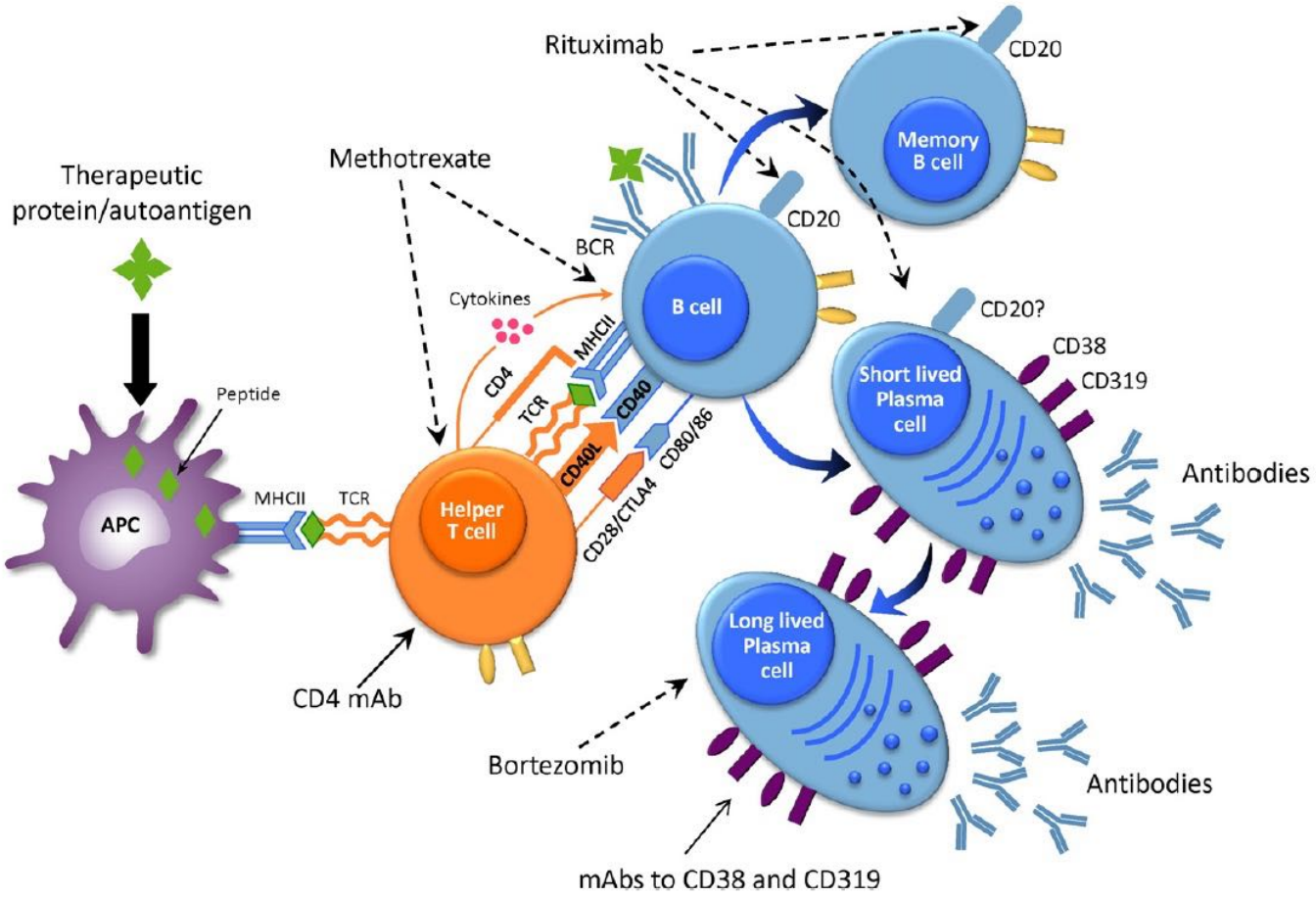
Cyclophosphamide (250 mg/m^2 IV

Rituximab (375 mg/m^2 IV)

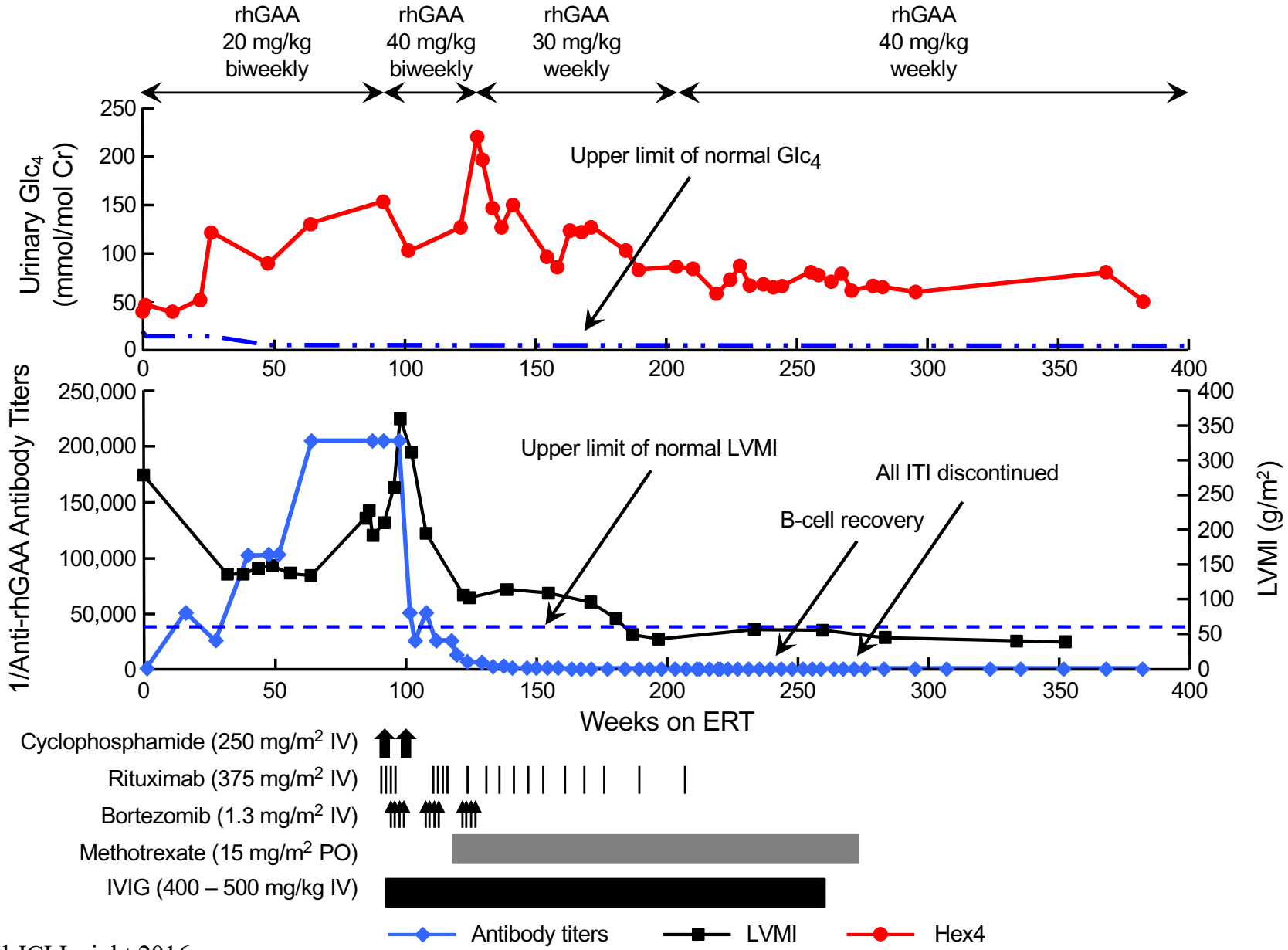
Methotrexate (15 mg/m^2 PO every other week)

IVIG (400-500 mg/kg IV monthly)

High Titer Sustained Antibody Responses are Mediated by Long Lived Plasma Cells: Unaffected by MTX/Rituximab



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



Kazi ZB et al JCI Insight 2016

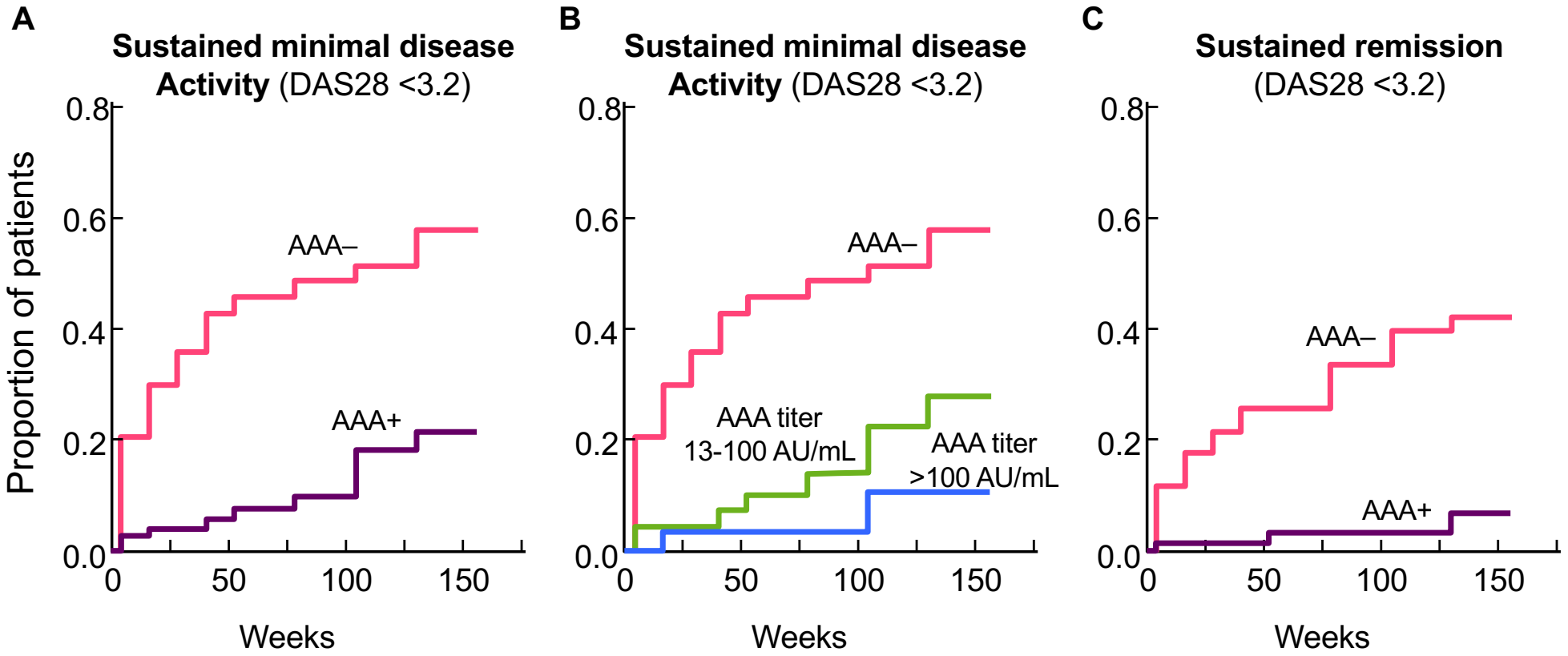
Applications for Prophylactic Tolerance Induction Strategy and Reversal of Antibody Responses Mediated by Pathogenic Antibody Secreting Plasma Cells

- Prevention of immune responses to therapeutic proteins
  - TNF inhibitory mAbs: frequent development of antibodies that neutralize efficacy
  - Enzyme replacement therapy in the setting of other lysosomal storage diseases in which antibodies are prominent, but clinical effect of ADA not known or investigated: **preponderance of data from multiple sources indicate antibody mediated interference in enzyme penetration of target tissue (Fabry Disease, MPS1)**

- Autoimmune diseases with pathogenic antibodies

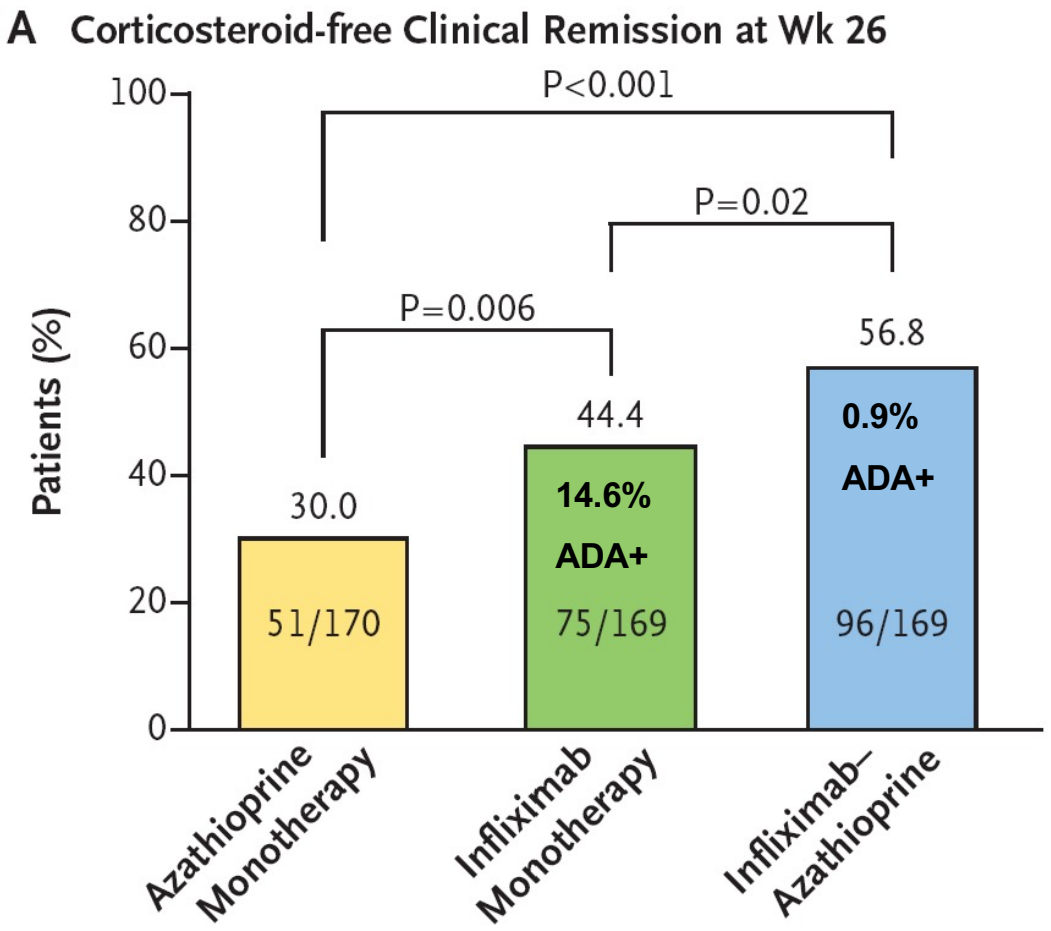
Antibodies to TNF mAbs Diminish Remission in RA: Would Immune Tolerance Induction be of Benefit?

(Bartelds G et al JAMA 2011)



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

(Colombel J-F et al NEJM 2010)



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: diminished with concomitant immune suppression)***.
- Are patients who receive concomitant immunesuppression, especially MTX, immune tolerant to TNF mAbs? Treg population specific for mAbs?
- Would short course of tolerance inducing agent (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.

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

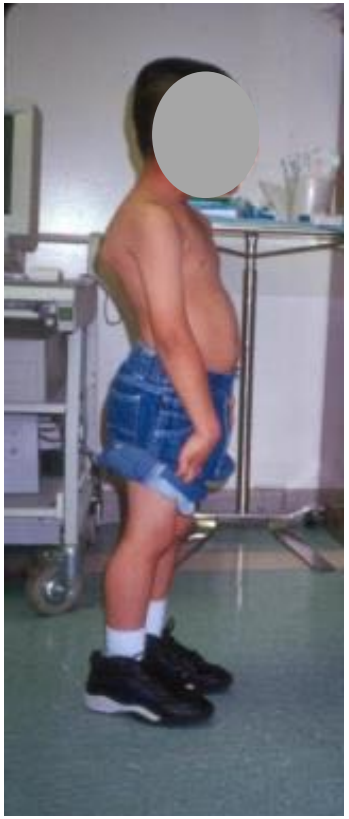
Diseases with pathogenic antibodies	Antibodies
SLE	Anti-DNA, anti-RNP
RA	RF anti-CCP
Vasculitis	Anti-myeloperoxidase, anti-proteinase 3
Myasthenia gravis	Anti-acetylcholine receptor
Hypothyroidism	Anti-thyroglobulin
Hyperthyroidism	Anti-TSH receptor
Vitiligo	Anti-melanocytes (melanin concentrating hormone 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 anemi	Anti-RBC
ITP	Anti-platelet

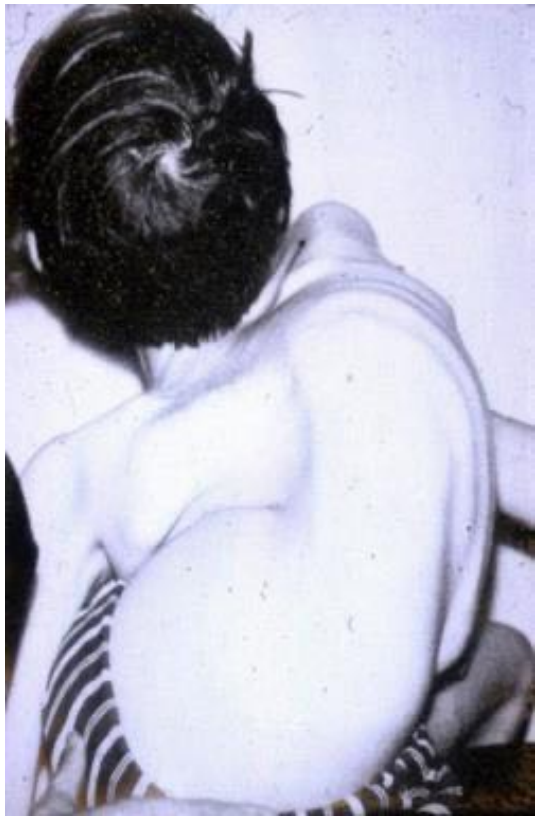
Rosenberg AS et al Clin Immunol 2016

Duchenne Muscular Dystrophy (DMD)

- DMD is an X-linked recessive, muscle-degenerative disorder.
- ***Most common inherited lethal disease of childhood: ~ 1:3500 boys are affected***.
- DMD is caused by mutations in the dystrophin gene, the majority of which result in the lack of functional protein.

- Early decrease in muscle strength
- Loss of ambulation by adolescence
- Early death (early-mid twenties)

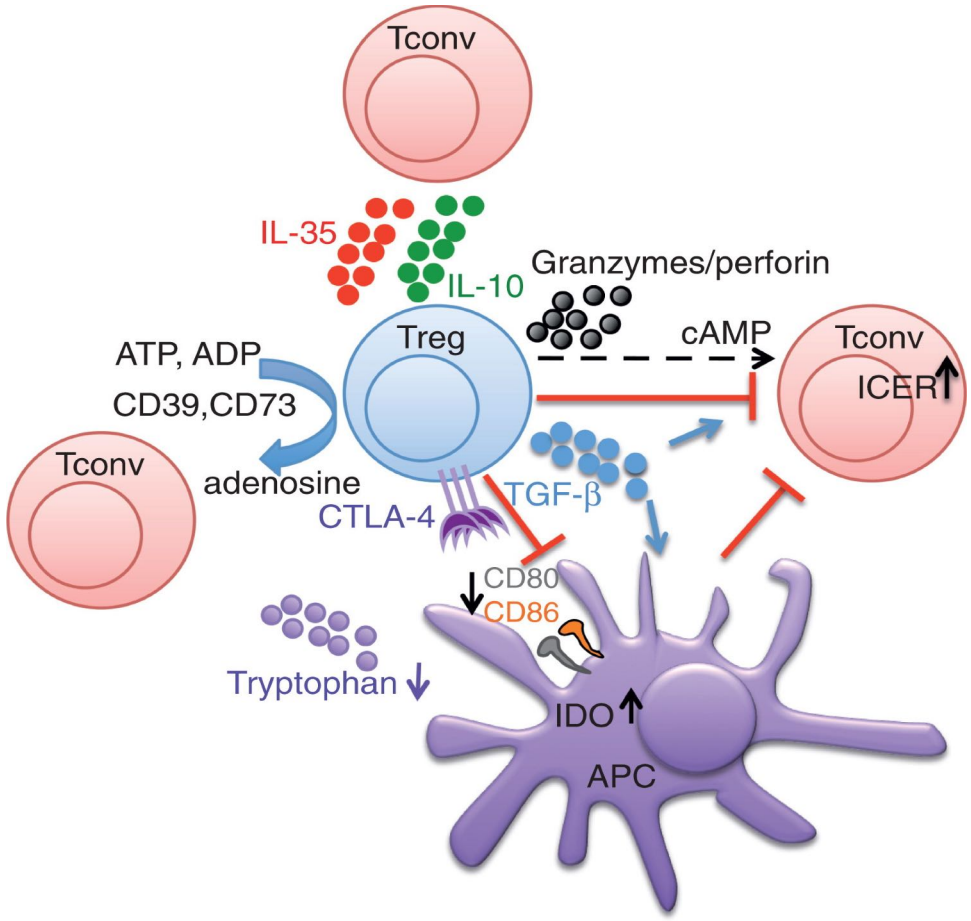




Strategies to Preclude Dystrophin Immunity Require Administration of Replacement Therapy in Optimized Setting

- Preclude or Eliminate Dystrophin Specific Immunity: Immune Tolerance Induction
  - Tolerance induction protocol for cell mediated (eg CD4+ and CD8+ T cells) responses rather than antibody mediated immune responses.
  - Tolerance induction in the context of transplantation and gene therapy are appropriate disease models
  - For gene therapy approaches to dystrophin replacement, must also consider immunity to vector (eg AAV) and to bacterial nucleases eg CRISPR/Cas9 therapies and whether tolerance induction needed vs transient immune suppression

Next Generation Immunotherapies for Autoimmunity, Transplantation, and Gene Therapy – T Regulatory cells

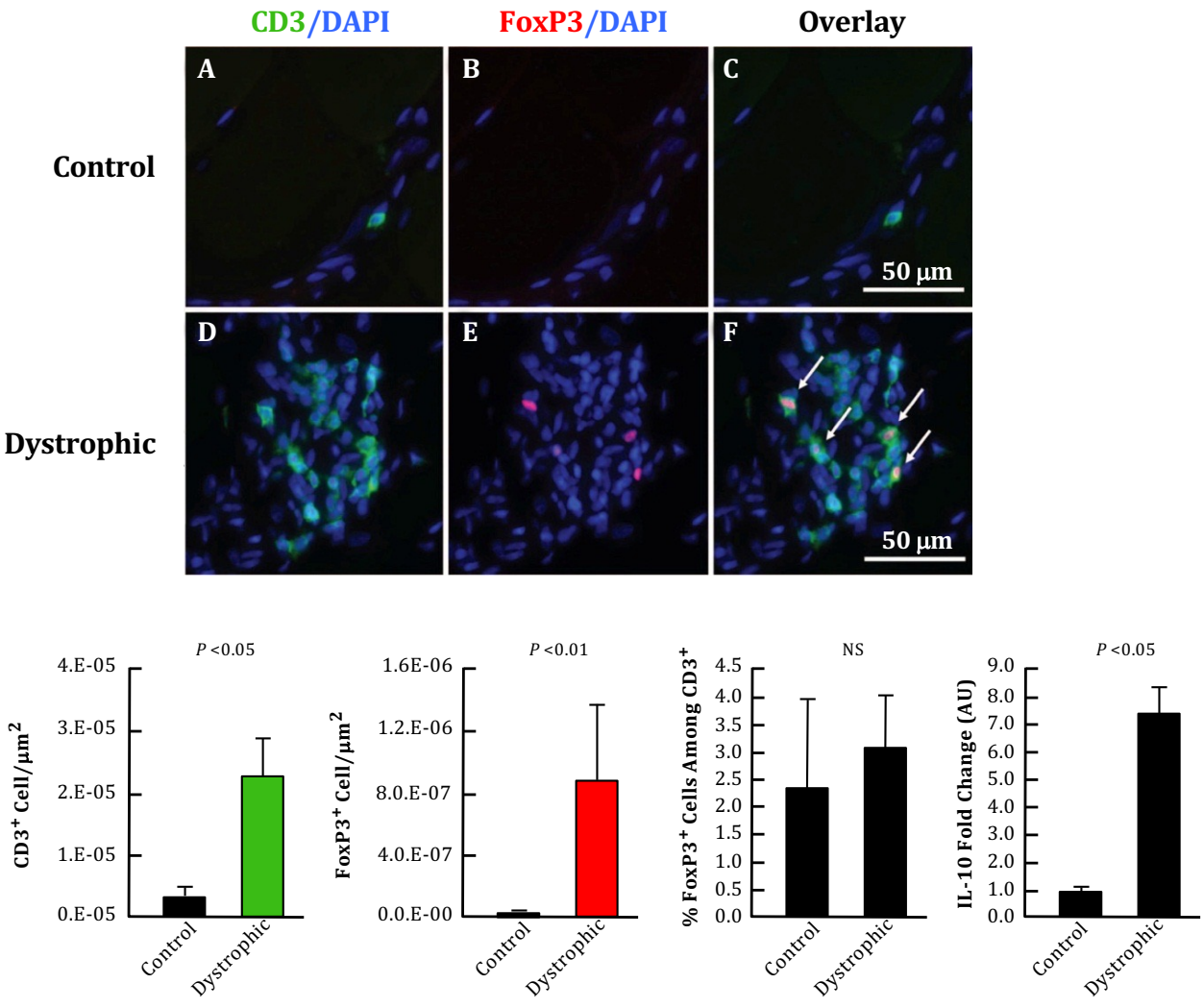


Bluestone JA et al *Expert Opinion on Therapeutic Targets* **2015**

Treg Therapy for Autoimmunity and Transplantation

- A living self-renewing therapeutic with immunologic memory (akin to tumor specific Tmemory cells)
- High specificity: avoidance of global immune suppression
- Induction of infectious tolerance via APCs
- Control a panoply of responses mediated by immune effector cells: conventional CD4+ and CD8+T cells, NK, NKT cells, B cells and APCs
- Use of varied immunosuppressive mechanisms depending on tissue microenvironment and stage of response:
  - Early stage: diminished activation by reduction of costimultory molecules (transendocytosis); sink for IL-2 and other cytokines
  - Later stage: Tregs proliferate, traffic and accumulate at site of inflammation: cytokine secretion, killing of APCs, ATPases

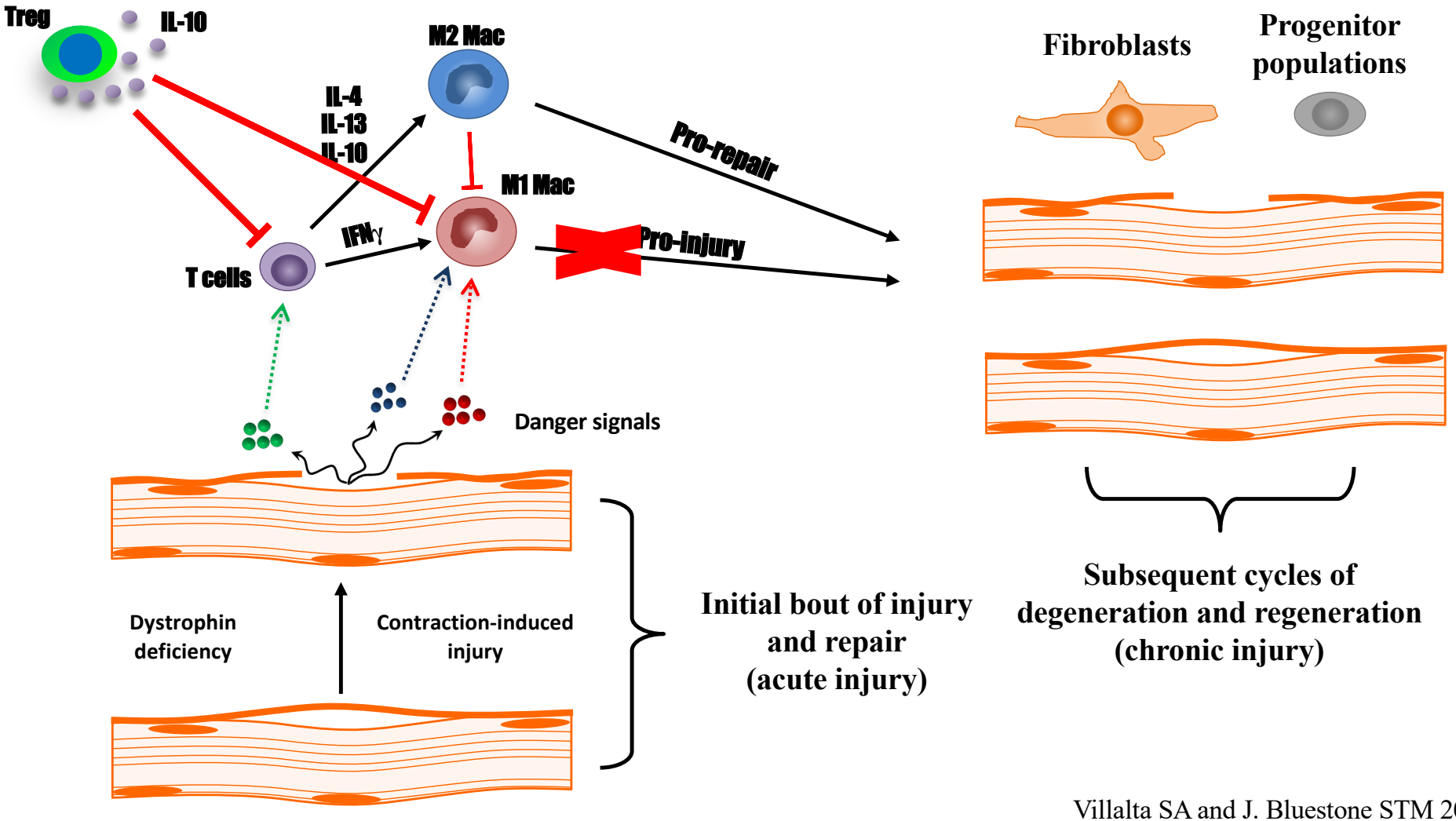
Tregs are Elevated in Muscle of Human Subjects with DMD/BMD



Villalta SA et al STM 2014

Role of Tregs in DMD: Suppression of Type 1 Pro-inflammatory Responses

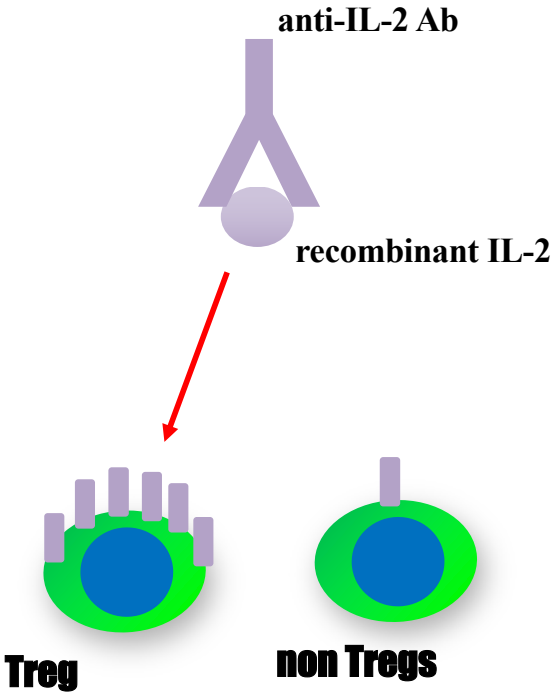
Evidence that Tregs modulate dystrophinopathy through the regulation of the immune response to injured muscle.

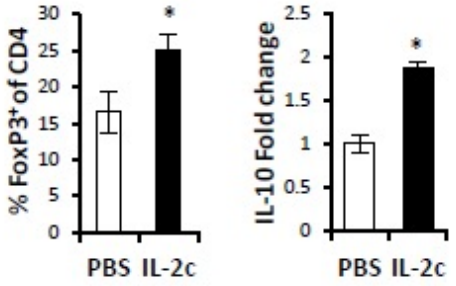


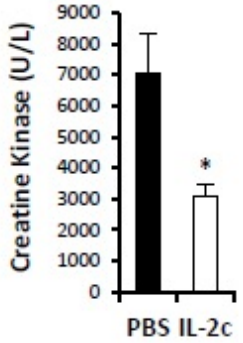
Villalta SA and J. Bluestone STM 2014

IL-2 Complex Treatments Increase Tregs and Reduce Muscle Inflammation and Injury in mdx mice

Low-dose IL-2c treatment preferentially induces Tregs *in vivo*







Villalta SA and J Bluestone STM 2014

Preclude or Eliminate Dystrophin Specific Immunity: Immune Tolerance Induction

- Tolerance protocols for transplantation as potentially applied to DMD:
  - Tregulatory cell promoting therapies: low dose IL-2; rapamycin, IL-10, anti-CD3 mAb, ATG, Tregitopes, Tolerizing DNA vaccines
  - Treg cellular therapy: per kidney transplant protocols; in phase 2 studies
  - Immunotolerizing/suppressive drug regimen per solid organ transplant

Coming to a Clinic Near You? Antigen Specific and Chimeric Antigen Receptor (CAR) Based Strategies for Tolerance Induction

1. Enrich and expand antigen specific Tregs with antigen/tetramer, etc.

2. Engineer specificity into polyclonal Tregs via transduction of specific T-cell receptor (TCR) or CAR (scFv), or even antigen (B-cell antibody receptor=BAR)

FDA Tools to Accelerate Clinical Development of Products Addressing Unmet Medical Needs

FDA Tools: Expedited Programs for Serious Conditions – Drugs and Biologics

Program	Qualifying Criteria: Serious condition and…	Features
Fast Track	-Nonclinical or clinical data demonstrate potential to meet an unmet medical need -Or, QIDP	-Actions to expedite development and review --E.g., meetings; rolling review
Breakthrough Therapy	-Preliminary clinical evidence indicates drug may demonstrate substantial improvement on a clinical significant endpoint over available therapies	-All Fast Track features -Intensive guidance on efficient drug development -Organizational commitment
Accelerated Approval	-Provides meaningful advantage over available therapies -demonstrates effect on surrogate or clinical endpoint that can be measured earlier than irreversible morbidity or mortality	-Approval based on a surrogate or intermediate clinical endpoint reasonably likely to predict clinical benefit
Priority Review	-Would provide a significant improvement in safety or effectiveness -Or, other qualifying programs	-Shorter review clock goal for marketing applications (6 mo vs 10 mo)

QIDP = qualifying infectious disease product;

Guidance for Industry

Expedited Programs for Serious Conditions – Drugs and Biologics

Available at:
http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM358301.pdf

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)

May 2014
Procedural

OMB Control No. 0910-0765
Expiration Date: 03/31/2017
See additional PRA statement in section X of this guidance.

Guidance for Industry

Codevelopment of Two or More New Investigational Drugs for Use in Combination

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

June 2013
Clinical Medical

Summary

- Expedited programs such as breakthrough and accelerated approval increasingly utilized to expedite access of transformative therapies to patients
- Increased proliferation of novel, adaptive master protocols, seamless designs
- Consideration of new approaches to response metrics, companion diagnostics, “real world” data

“…We choose to go to the moon in this decade and do the other things, not because they are easy, but because they are hard, because that goal will serve to organize and measure the best of our energies and skills, because that challenge is one that we are willing to accept, one we are unwilling to postpone, and one which we intend to win…”

JFK Rice University 1962

“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

“If not now, then when”?

Hillel

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