Immune Tolerance Induction in Enzyme Replacement Therapy for Lysosomal Storage Diseases: Optimizing Therapeutic Outcome with Preventive Strategies

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Risk of Immune Response Across the Spectrum of Lysosomal Storage Diseases: What do we Know about the Consequences?

Knowledge about likelihoods

- Strong basis for probabilities
- No basis for probabilities

Knowledge about Consequences

Consequences well-defined
- Pompe Disease

Consequences poorly-defined
- Fabry Disease, MPS1

Risk (I)

Ambiguity (III)

Incertitude

Uncertainty (II)

Ignorance (IV)

(modified from Stirling and Ghee 2002)
Acting on Immunogenicity Risk Assessment

- Severity of consequences of the immune response to therapeutic proteins determines course of action:
  - when consequences are life threatening, tolerance induction is indicated
  - when life threatening immune responses can be predicted based on genetic mutation or CRIM status, prophylactic tolerance protocols are indicated: incur less risk than either poorly treated lysosomal storage disease or more prolonged therapeutic immune suppressive tolerance induction regimens
  - tolerance induction should be strongly considered when a preponderance of evidence indicates that the immune response is diminishing the efficacy of effective (but not necessarily life saving) therapeutics
  - risks associated with tolerance regimens and impact of tolerance regimen on underlying disease should be considered
Consequences of the Immune Response to Protein Therapeutics for which Immune Tolerance Induction is Indicated

- **Abrogation of the efficacy of life saving therapeutics by neutralizing antibodies or sustained high titer antibodies**
  - Coagulation and Enzyme Replacement Therapies in CRIM negative patients

- **Anaphylaxis to life saving therapeutic proteins**
  - Highly efficacious proteins of non-human origin
  - ERT, Factor IX in hemophilia B

- **Neutralization of endogenous factor with unique function resulting in cellular or critical factor deficiency syndrome**
  - Thrombopoietin: thrombocytopenia
  - Erythropoietin: pure red cell aplasia
  - GM-CSF: acquired pulmonary alveolar proteinosis
Antibody May Block ERT Target Cell Entry and Catalytic Activity or Facilitate Enzyme Uptake in Nontarget Cells
Pompe Disease

- Infantile Pompe disease (IPD) – Lysosomal Storage Disease (LSD) resulting from a deficiency of lysosomal acid alpha glucosidase (GAA).
- Can be a rapidly fatal disease primarily affecting cardiac and skeletal muscle: death within the first 2 years of life from cardiac and respiratory failure.
- Because of rapid progression, efficacy of Enzyme Replacement Therapy (ERT) and effects of immune responses to ERT are clear (heart failure, weakness, requirement for invasive ventilation), as compared to slowly progressive LSDs with more distant endpoints.
- Recombinant human GAA (rhGAA, Myozyme and Lumizyme®) is currently the only available therapeutic. No bypass or salvage therapies.
CRIM Negative and A Subset of CRIM Positive Patients Mount High Titer/Sustained Antibody Responses to ERT
(Kishnani PS et al 2011)
High Titer Antibody Response, not CRIM Status Per Se, Confers Negative Clinical Outcome in ERT-Treated Patients with Pompe Disease

(Kishnani PS et al 2011)
Clinical Scenario 1: Treatment Naïve Patient
(P. Kishnani ITN, May 2007)

- Birth weight 7lb8oz, cleft lip and palate.
- At age 5 weeks: respiratory symptoms, cardiomegaly, hypertrophic cardiomyopathy 166g/m², SVT.
- Diagnosis of Pompe made based on absent GAA, homozygous for R854stop, CRIM negative on skin western
- Need for immediate treatment due to cardiac and respiratory failure
Potential Approaches to Immune Tolerance Induction

• Ideal: Approaches that are highly antigen specific with consequent preservation of global immunity
  – Protein therapeutics: linked tolerance via known tolerogens, e.g., Fc moieties; tolerance (e.g., Factor VIII) protocols based on dose escalation and frequency
  – Cellular therapy: induction/administration of antigen specific regulatory T cells (Tregs); mesenchymal stem cells;
  – Gene therapy: some success in patients with hemophilia B; successful in mouse models of Pompe Disease-liver targeting essential
  – Oral tolerance

• Antigen specific approaches may be problematic for clinical settings requiring immediate treatment or in clinical settings in which significant end organ damage may occur during a prolonged course of tolerance induction
  – Antigen specific approaches may take weeks-months for induction of durable immune tolerance
  – End organ damage may potentially progress/become irreversible during prolonged tolerance induction protocols
  – In situations in which clinical treatment and thus, tolerance induction must begin urgently because of deteriorating clinical status, therapeutic options are more limited
Tolerance Induction Strategies: Targeting Components of the Adaptive Immune Response

**Targeted therapeutic protein: tolerogenic DCs**

**Methotrexate**

**Rituximab**

**CD20**

**Memory B cell**

**Short lived Plasma cell**

**Long lived Plasma cell**

**CD4mAb**

**Helper T cell**

**B cell**

**CD20?**

**Antibodies**

**DC**

**Peptide**

**TCR**

**MHCII**

**Cytokines**

**Non-depleting CD4mAb**

**Bortezomib**
Prophylactic ITI Protocol

- **Wk0**: Alglucosidase alfa (20 mg/kg every other week)
- **Wk1**: Rituximab IV (375 mg/m²; if BSA<0.5 m², 12.5 mg/kg)
- **Wk2**: Methotrexate SC (0.4 mg/kg)
- **Wk3**: IVIG (400-500 mg/kg)

Banugaria S et al PlosOne 2013
Efficacy of Prophylactic ITI + ERT

Banugaria SG et al PlosOne 2013
Improved Ventilator Free Survival with ITI + ERT

Banugaria SG et al PlosOne 2013
Prophylactic Approach to Tolerance Induction

- Limited experience, but excellent safety profile: one case of infection (bronchitis) which cleared rapidly.
- Two patients with titers $\geq 1:6400$ required a second course: decreased titer in one; stable titer in second.
- One patient died of progressive Pompe Disease not related to tolerance induction.
- **Recommended protocol for CRIM negative Pompe Disease at onset of enzyme replacement therapy**
- Given the safety profile, should this protocol be recommended for CRIM positive patients until we can predict those who will mount high titer?
Clinical Scenario 2: High Sustained Antibody Titers
(P. Kishnani ITN, May 2007)

- At age 5 weeks: respiratory symptoms, cardiomegaly, hypertrophic cardiomyopathy 166g/m², SVT.
- Diagnosis of Pompe made based on absent GAA: Homozygous for R854stop; CRIM negative
- First Myozyme (rhGAA) infusion at age 6 weeks. Dosed at 20mg/kg every 2 weeks
- Patient develops high sustained antibody titers and clinically deteriorates: titer >1:25,000 despite treatment with immune suppressive agents

Information received from Children’s Hospitals and Clinics of Minnesota
Requirement for Intensive and Prolonged Immune Suppression to Eliminate Antibody Response in Patients with High Sustained Antibody Titers

(Kishnani PS et al 2012)

1/Anti-rhGAA IgG Antibody Titer

Weeks on ERT

Left Ventricular Mass Index (g/m²)

Rituximab (375 mg/m²IV)
Methotrexate (15 mg/m²SC)
Bortezomib (1.3 mg/m²IV)
IVIG (400-500 mg/kg IV monthly)
Confirmed CRIM negative

ITI + ERT

Monitor anti-rhGAA IgG antibodies, CD19% and clinical progress

No or low antibodies
(<6,400) and CD19% recovery at ≥ 5 months

Increasing antibodies
(≥6,400) at two or more time points and CD19% recovery at ≥ 5 months

Monitor antibodies and clinical outcome parameters
Repeat ITI and monitor antibodies, CD19% and clinical progress
ITI with plasma cell targeting agent

No or low antibodies
(<6,400) and CD19% recovery at ≥ 5 months of last ITI
Increasing Antibodies
(≥6,400) at two or more time points and CD19% recovery at ≥ 5 months after last ITI

Kishnani PS et al
PLoS One 2013
Tolerance Induction Indicated when the Consequence of the Immune Response to Protein Therapeutics is Fatality/Severe Morbidity

• Neutralizing antibodies or high titer antibodies that neutralize efficacy of life saving therapeutics
  – Enzyme and coagulation factor replacement therapies in protein knock out (CRIM negative) phenotype

• Anaphylaxis to life saving therapeutic proteins
  – Highly efficacious proteins of non-human origin
  – Human proteins in CRIM negative patients: e.g., ERT (rare), Factor IX in hemophilia B

• Neutralization of endogenous factor with unique function resulting in cellular or critical factor deficiency syndrome
  – Thrombopoietin: thrombocytopenia
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Management of Anaphylaxis to Factor IX: Lessons for IPD

• Avoidance:
  – Factor IX bypass therapy; no bypass therapy for Pompe Disease

• Desensitization: not an appropriate strategy for Pompe Disease in absence of bypass therapy

• Tolerance Induction
  – New approaches to Tolerance Induction
    • Anti-CD20 mAb
    • Anti-IgE mAb should be considered for IgE positive patients with anaphylaxis
    • Gene therapy
Anti-IgE for Treatment of IgE Mediated Anaphylaxis to a Therapeutic Enzyme
(Rohrbach et al J. Inherit Metab Dis 2010)

- Patient with IPD on ERT experienced persistent life-threatening anaphylaxis episodes that were not controlled with corticosteroids, antihistamines, or decreased infusion rate.
  - A scratch skin test for Myozyme tested positive
  - IgE positive for ERT

- Treatment regimen was optimized by adding omalizumab, a recombinant monoclonal antibody against IgE.
  - Patient continued to receive ERT
  - Treatment for anaphylaxis weaned with the exception of omalizumab.
    - rhGAA specific IgG antibody titers were measured routinely every 4–5 months and were always <1:800 (low).

- **Continuation of omalizumab necessary? Did anti-IgE treatment preclude generation of high titer IgG response?**
What is the Evidence that the Immune Response to ERT for other Lysosomal Storage Diseases Affects Safety and Efficacy?

• Clinical manifestations, effect of ERT and effect of immune response to ERT are very clear in Pompe Disease with loss of motor milestones and cardiac failure;

• The effects of ERT on clinical manifestations and effect of immune response to ERT in more slowly progressive LSDs are not clear in many cases. Clinical endpoints for some are only reached after many years of disease: e.g., MPS1

• Validated biomarkers that predict clinical efficacy of ERT and reveal impact of immune response to ERT are lacking for most LSDs
Ambiguity
(modified from Stirling and Ghee 2002)

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MPS 1

• Deficiency of $\alpha$-L-iduronidase leading to accumulation of heparan sulfate and dermatan sulfate glycosaminoglycans in lysosomes

• Spectrum ranging in severity from Hurler’s (severe) to Scheie (attenuated) with Hurler-Scheie intermediate in spectrum

• Hurler’s presents early (birth-2 years) and rapidly progresses: mental retardation, dwarfism, coarse facies, dysostosis multiplex and hepatosplenomegaly; death within a decade if untreated

• Scheie: onset delayed; little or no neuronal involvement

• Treatment
  – ERT: a-L-iduronidase (Laronidase)
  – Allogeneic BMT
CRIM Negative MPS I Patients do not Tolerize Over Time

• Under 5 Study in Hurler (CRIM negative) patients
  – Stronger immune responses
  – Some decrease in urinary GAG reduction
  – Decline in titers but not tolerant
Most CRIM Negative Patients with MPS 1 do not Tolerize Over Time

(Genzyme, unpublished)
Evidence that Antibody Response Affects Optimal Activity of ERT in MPS I

- Correlation of disease biomarkers with antibody response in MPS I patients

- Immune tolerance to ERT improves tissue penetration of enzyme, substrate reduction, and improved histopathology (with higher treatment dose) in CRIM negative MPS I dogs
Antibodies to Laronidase Associated with Increased Substrate Levels

(Wraith JE et al 2007)

% Reduction in Urinary GAG

Antibody Titer (log scale)

Higher level: > 1600
Lower level: < 1600

below median < 63.3 %
above median > 63.3 %

100 U/kg, MPS I H
200 U/kg, MPS I H
MPS 1 Dogs Have Robust Antibody Response to α-L-Iduronidase but can be Tolerized

(Kakkis E et al 2004)
Improved Target Tissue Penetration, Substrate Reduction and Histopathology in Tolerant MPS I Dogs: Relevance to Human MPS1?

(Dickson P et al 2008)

A

Liver  
Spleen  
Lymph node  
Renal cortex  
Renal medulla  
Lung  
Heart valve  
Myocardium  
Synovium  
Cartilage (rib)

Iduronidase level (U/mg protein)

0 5 10 15 20 25 30 35 40

Nontol 0.58 mg/kg

B

Liver  
Spleen  
Lymph node  
Renal cortex  
Renal medulla  
Lung  
Heart valve  
Myocardium  
Synovium  
Cartilage (rib)

% Change vs. Nontol 0.58 mg/kg

-200 0 200 400 600 2000

Tol 0.58 mg/kg
Fabry Disease

- X-linked LSD: deficiency of α-galactosidase
- Glycosphingolipids (globotriaosylceramide-Gb3) accumulate and alter the morphology and function of many cell types, including vascular endothelium, visceral parenchyma, and some central and peripheral neurons.
- Highly heterogeneous disease course with clinical manifestations varying widely in onset and severity among patients. Initial symptoms include acroparesthesia, anhidrosis, and angiokeratoma but renal insufficiency, cardiac involvement, and cerebrovascular complications commonly arise in mid-adulthood, reducing life expectancy
- rh-α galactosidase received accelerated approved in 2003 based on a surrogate endpoint: clearance of Gb3 from renal vascular endothelium
Most Male Patients with Fabry Disease Fail to Tolerize to rh-α-galactosidase with Continued Treatment

(Wilcox WR et al 2012)

- Males (N=571)
  - Seroconverted, not tolerated (n=369) 65%
  - Seronegative (n=155)
  - Seroconverted, then tolerated (n=47)

- Females (N=251)
  - Seroconverted, not tolerated (n=13) 5%
  - Seronegative (n=220)
  - Seroconverted, then tolerated (n=18) 32%
Evidence that Antibody Response Affects Optimal Activity of ERT in Fabry Disease

• Correlation of disease biomarkers with antibody response in patients with Fabry Disease.

• Immune tolerance to ERT improves tissue penetration and substrate reduction in patients and animal models.
Correlation of Antibody Titer with Substrate Accumulation in Dermal Capillary Endothelium: Reflects Accumulation in Critical Target Endothelia?  
(Benichou et al 2009)

![Graph showing correlation between antibody titer and substrate accumulation](image-url)
Dosage Level of ERT Reveals Strong Effect of Antibodies on Substrate Excretion

Mean urine Gb-3 (μg/mg creatinine)

- Baseline
- 1.0 mg/kg period (weeks 0-24)
- 0.3 mg/kg period (weeks 25-96)

* p < 0.05, different from baseline

Lubanda JC et al 2009
Neutralizing Antibody Positivity Correlates with Higher Substrate Excretion Levels in Patients with Fabry

Rombach et al 2012
Antibodies to α-Galactosidase Diminish Enzyme Penetration of Target Tissues in KO Mice

(Ohashi et al 2008)

**Kidney**

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<th>Injection mixture</th>
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<tr>
<td>Enzyme (-)</td>
<td>0</td>
</tr>
<tr>
<td>Seropositive</td>
<td>*</td>
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<td>Seronegative</td>
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**Lung**

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• Preponderance of evidence indicates that moderate to high titer antibody response to ERT diminishes tissue penetration, reduces clearance of substrate, and thus, diminishes efficacy of ERT.

• Given the safety of prophylactic tolerance induction regimens, strong consideration should be given to prophylactic tolerance induction in patients with LSDs at high risk of mounting such responses to ERT.
• Develop disease-specific biomarkers and endpoints to assess the effect of anti-drug antibodies and immune tolerance induction on efficacy

• Prophylactic immune tolerance induction should be strongly considered in patients who are at risk of developing immune responses to ERT
Conclusions and Questions

• A preponderance of evidence indicates that persistent, moderate to high titer or neutralizing antibody responses interfere with ERT uptake and/or activity in critical target tissues and thus very likely diminish efficacy.

• Prophylactic immune tolerance induction allows for unimpeded ERT activity, diminishing tissue damage, and reducing the intensity and duration of immune suppression associated with therapeutic tolerance induction protocols.

• Given the very favorable safety experience with the prophylactic tolerance induction protocol in Pompe (worst case scenario), should tolerance induction be considered in all CRIM negative and high risk CRIM positive patients with LSDs?

• Immune tolerance to ERT has additional potential benefits:
  – Reducing or eliminating immune response to potentially more highly efficacious therapies: biobetter ERT, gene therapy and exon skipping approaches to therapy
  – Establish tolerance to enzyme to diminish immunogenicity and enhance engraftment in the setting of transplant
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Risk of Developing Additional White Matter Lesions Correlates with Substrate LysoGb3 Level

Rombach et al 2012