

UCLA

UCLA Previously Published Works

Title

Immune response to enzyme replacement therapies in lysosomal storage diseases and the role of immune tolerance induction

Permalink

<https://escholarship.org/uc/item/59n944ht>

Journal

Molecular Genetics and Metabolism, 117(2)

ISSN

1096-7192

Authors

Kishnani, Priya S
Dickson, Patricia I
Muldowney, Laurie
[et al.](#)

Publication Date

2016-02-01

DOI

10.1016/j.ymgme.2015.11.001

Peer reviewed

Accepted Manuscript

Immune response to enzyme replacement therapies in lysosomal storage diseases and the role of immune tolerance induction

Priya S. Kishnani, Patricia I. Dickson, Laurie Muldowney, Jessica J. Lee, Amy Rosenberg, Rekha Abichandani, Jeffrey A. Bluestone, Barbara K. Burton, Maureen Dewey, Alexandra Freitas, Derek Gavin, Donna Griebel, Melissa Hogan, Stephen Holland, Pranoot Tanpaiboon, Laurence A. Turka, Jeanine J. Utz, Yow-Ming Wang, Chester B. Whitley, Zoheb B. Kazi, Anne R. Pariser

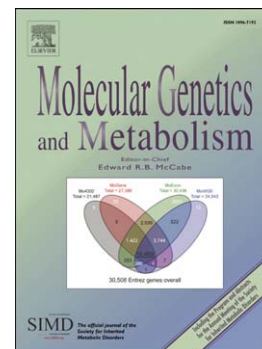
PII: S1096-7192(15)30071-8
DOI: doi: [10.1016/j.ymgme.2015.11.001](https://doi.org/10.1016/j.ymgme.2015.11.001)
Reference: YMGME 5971

To appear in: *Molecular Genetics and Metabolism*

Received date: 14 September 2015
Revised date: 2 November 2015
Accepted date: 3 November 2015

Please cite this article as: Kishnani, P.S., Dickson, P.I., Muldowney, L., Lee, J.J., Rosenberg, A., Abichandani, R., Bluestone, J.A., Burton, B.K., Dewey, M., Freitas, A., Gavin, D., Griebel, D., Hogan, M., Holland, S., Tanpaiboon, P., Turka, L.A., Utz, J.J., Wang, Y.-M., Whitley, C.B., Kazi, Z.B. & Pariser, A.R., Immune response to enzyme replacement therapies in lysosomal storage diseases and the role of immune tolerance induction, *Molecular Genetics and Metabolism* (2015), doi: [10.1016/j.ymgme.2015.11.001](https://doi.org/10.1016/j.ymgme.2015.11.001)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Immune Response to Enzyme Replacement Therapies in Lysosomal Storage Diseases and the Role of Immune Tolerance Induction[☆]

Authors : Priya S. Kishnani^a, Patricia I. Dickson^b, Laurie Muldowney^c, Jessica J. Lee^c, Amy Rosenberg^d, Rekha Abichandani^e, Jeffrey A. Bluestone^f, Barbara K. Burton^g, Maureen Dewey^c, Alexandra Freitas^h, Derek Gavin^h, Donna Griebel^c, Melissa Hoganⁱ, Stephen Holland^j, Pranoot Tanpaiboon^k, Laurence A. Turka^l, Jeanine J. Utz^m, Yow-Ming Wangⁿ, Chester B. Whitley^m, Zoheb B. Kazi^a, Anne R. Pariser^{o*}

^aDivision of Medical Genetics, Department of Pediatrics, Duke University Medical Center, Durham NC 27710, USA

^bDivision of Medical Genetics, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA 90505-2006, USA

^cDivision of Gastroenterology and Inborn Errors of Metabolism Products, Office of New Drugs, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), Silver Spring, MD 20993-0002, USA

^dDivision of Therapeutic Proteins, Office of Biotechnology Products, CDER, FDA, Silver Spring, MD 20993-0002, USA

^eShire, Lexington, MA 02421, USA

^fDiabetes Center, University of California, San Francisco San Francisco, CA 94143-0540, USA

Abbreviations: Ab, antibody; ADA, anti-drug antibodies; Ag, antigen; BiPAP, bilevel positive airway pressure; CDER, Center for Drug Evaluation and Research CNS; central nervous system; CRIM, cross-reactive immunologic material; DGIEP, Division of Gastroenterology and Inborn Errors of Metabolism; ERT, enzyme replacement therapy; FcR, Fc receptor; FDA, Food and Drug Administration; GAA, acid alpha glucosidase; GAG, glycosaminoglycans; HLA, human leukocyte antigen; HSAT, high and sustained anti-rhGAA IgG antibody titers; HSCT, hematopoietic stem cell transplantation; IDS, iduronate-2-sulfatase; IPD, infantile-onset Pompe disease; IRR, infusion-related reaction; IT, intrathecal; ITI, immune tolerance induction; IV, intravenous; IVIG, intravenous immune globulin; KO, knock-out; LSD, lysosomal storage disorder; LVMI, left ventricular mass index; mAb, monoclonal antibody; MHC, major histocompatibility complex; MPS, mucopolysaccharidosis; M6P, mannose-6-phosphate; NORD, National Organization for Rare Disorders; rhGAA, recombinant human acid alpha glucosidase; Tregs, regulatory T cells; 6MWT, six-minute walk test.

[☆]The opinions presented herein are those of the authors and do not reflect the positions of all participants nor the institutions they represent.

*Corresponding author: Anne R. Pariser, Office of Translational Sciences, Center for Drug Evaluation and Research, Food and Drug Administration, 10903 New Hampshire Avenue, WO21-4607, Silver Spring, MD 20993-0002, USA, 301-796-0968, anne.pariser@fda.hhs.gov

Email addresses: priya.kishnani@duke.edu (PS Kishnani), pdickson@labiomed.org (PI Dickson), laurie.muldowney@fda.hhs.gov (L Muldowney), jessica.j.lee@fda.hhs.gov (JJ Lee), amy.rosenberg@fda.hhs.gov (A Rosenberg), rabichan@shire.com (R Abichandani), jeff.bluestone@ucsf.edu (J Bluestone), bburton@luriechildrens.org (BK Burton), maureen.dewey@fda.hhs.gov (M Dewey), afreitas@rarediseases.org (A Freitas), dgavin@rarediseases.org (D Gavin), donna.griebel@fda.hhs.gov (D Griebel), melissa@savingcase.com (M Hogan), steve.holland@thomsonreuters.com (S Holland), ptanpaib@childrensnational.org (P Tanpaiboon), lturka@partners.org (L Turka), utz002@umn.edu (J Utz), yowming.wang@fda.hhs.gov (YM Wang), whitley@umn.edu (CB Whitley), zoheb.kazi@dm.duke.edu (Z Kazi), anne.pariser@fda.hhs.gov (AR Pariser)

^eNorthwestern University Feinberg School of Medicine and the Ann & Robert H. Lurie Children's Hospital, Chicago, IL 60611

^hNational Organization for Rare Disorders, Washington, DC 20036

ⁱSaving Case & Friends, Inc., a Hunter Syndrome Research Foundation, Thompson's Station, TN 37179

^jNational MPS Society, Durham, NC 27709

^kChildren's National Medical Center, Washington, DC 20010

^lCenter for Transplantation Sciences, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02129

^mUniversity of Minnesota. Masonic Children's Hospital, Minneapolis, MN 55455, USA

ⁿDivision of Clinical Pharmacology III, Office of Clinical Pharmacology, Office of Translational Sciences (OTS), CDER, FDA, Silver Spring, MD 20993-0002, USA

^oOTS, CDER, FDA, Silver Spring, MD 20993-0002, USA

Abstract word count: 282

Word count: 11,621

Number of references: 61

Number of tables and figures: 12

Key words: Immune tolerance, lysosomal storage diseases, enzyme replacement therapy, inborn errors of metabolism, neutralizing antibodies, orphan drugs, rare diseases

Revised, Date: November 2, 2015

Abstract

The US Food and Drug Administration (FDA) and National Organization for Rare Disease (NORD) convened a public workshop titled “Immune Responses to Enzyme Replacement Therapies: Role of Immune Tolerance Induction” to discuss the impact of anti-drug antibodies (ADA) on efficacy and safety of enzyme replacement therapies (ERTs) intended to treat patients with lysosomal storage diseases (LSDs). Participants in the workshop included FDA staff, clinicians, scientists, patients, industry, and advocacy group representatives. The risks and benefits of implementing prophylactic immune tolerance induction (ITI) to reduce the potential clinical impact of antibody development were considered. Complications due to immune responses to ERT are being recognized with increasing experience and lengths of exposure to ERTs to treat several LSDs. Strategies to mitigate immune responses and to optimize therapies are needed. Discussions during the workshop resulted in the identification of knowledge gaps and future areas of research, as well as the following proposals from the participants:

Abbreviations: Ab, antibody; ADA, anti-drug antibodies; Ag, antigen; BiPAP, bilevel positive airway pressure; CDER, Center for Drug Evaluation and Research CNS; central nervous system; CRIM, cross-reactive immunologic material; DGIEP, Division of Gastroenterology and Inborn Errors of Metabolism; ERT, enzyme replacement therapy; FcR, Fc receptor; FDA, Food and Drug Administration; GAA, acid alpha glucosidase; GAG, glycosaminoglycans; HLA, human leukocyte antigen; HSAT, high and sustained anti-rhGAA IgG antibody titers; HSCT, hematopoietic stem cell transplantation; IDS, iduronate-2-sulfatase; IPD, infantile-onset Pompe disease; IRR, infusion-related reaction; IT, intrathecal; ITI, immune tolerance induction; IV, intravenous; IVIG, intravenous immune globulin; KO, knock-out; LSD, lysosomal storage disorder; LVMI, left ventricular mass index; mAb, monoclonal antibody; MHC, major histocompatibility complex; MPS, mucopolysaccharidosis; M6P, mannose-6-phosphate; NORD, National Organization for Rare Disorders; rhGAA, recombinant human acid alpha glucosidase; Tregs, regulatory T cells; 6MWT, six-minute walk test.

★The opinions presented herein are those of the authors and do not reflect the positions of all participants nor the institutions they represent.

*Corresponding author: Anne R. Pariser, Office of Translational Sciences, Center for Drug Evaluation and Research, Food and Drug Administration, 10903 New Hampshire Avenue, WO21-4607, Silver Spring, MD 20993-0002, USA, 301-796-0968, anne.pariser@fda.hhs.gov

Email addresses: priya.kishnani@duke.edu (PS Kishnani), pdickson@labiomed.org (PI Dickson), laurie.muldowney@fda.hhs.gov (L Muldowney), jessica.j.lee@fda.hhs.gov (JJ Lee), amy.rosenberg@fda.hhs.gov (A Rosenberg), rabichan@shire.com (R Abichandani), jeff.bluestone@ucsf.edu (J Bluestone), bburton@luriechildrens.org (BK Burton), maureen.dewey@fda.hhs.gov (M Dewey), afreitas@rarediseases.org (A Freitas), dgavin@rarediseases.org (D Gavin), donna.griebel@fda.hhs.gov (D Griebel), melissa@savingcase.com (M Hogan), steve.holland@thomsonreuters.com (S Holland), ptanpaib@childrensnational.org (P Tanpaiboon), lturka@partners.org (L Turka), utz002@umn.edu (J Utz), yowming.wang@fda.hhs.gov (YM Wang), whitley@umn.edu (CB Whitley), zoheb.kazi@dm.duke.edu (Z Kazi), anne.pariser@fda.hhs.gov (AR Pariser)

(1) systematic collection of longitudinal data on immunogenicity to better understand the impact of ADA on long-term clinical outcomes; (2) development of disease-specific biomarkers and outcome measures to assess the effect of ADA and ITI on efficacy and safety; (3) development of consistent approaches to ADA assays to allow comparisons of immunogenicity data across different products and disease groups, and to expedite reporting of results; (4) establishment of a system to widely share data on antibody titers following treatment with ERTs; (5) identification of components of the protein that are immunogenic so that triggers and components of the immune responses can be targeted in ITI; and (6) consideration of early ITI in patients who are at risk of developing clinically relevant ADA that have been demonstrated to worsen treatment outcomes.

Immune Response to Enzyme Replacement Therapies in Lysosomal Storage Disorders and the Role of Immune Tolerance Induction

1. Introduction

On June 9, 2014, the U.S. Food and Drug Administration (FDA)'s Center for Drug Evaluation and Research (CDER), Division of Gastroenterology and Inborn Errors Products (DGIEP), and National Organization for Rare Disorders (NORD) hosted a workshop titled "Immune Responses to Enzyme Replacement Therapies: Role of Immune Tolerance Induction" [1]. Attendees included approximately 220 participants from academia and clinical institutions, industry, patients, advocacy organizations, and regulatory and other governmental agencies. The goals of the workshop were to discuss the impact of anti-drug antibodies (ADA) on efficacy and safety of enzyme replacement therapies (ERTs) intended to treat patients with lysosomal storage diseases (LSDs), and to discuss the potential risks and benefits of implementing prophylactic immune tolerance induction (ITI) to reduce the potential clinical impact of ADA development.

The workshop was organized into 2 major sessions: 1) Immune responses to ERT, and 2) the role of ITI in ERT. State-of-the-art overviews, original research, case studies, and patient/parent experiences were presented, and robust discussions by panels of experts representing all of the stakeholder communities were held. This article is a summary of the proceedings from the workshop and outlines the proposals from the participants for addressing knowledge gaps and identifying future areas for research.

2. Background

Lysosomal storage diseases (LSDs) are a heterogeneous group of as many as 70 autosomal or X-linked genetic diseases in which a single gene mutation leads to a deficiency in or absence of specific lysosomal enzyme activity [2, 3, 4]. These deficiencies result in the accumulation of macromolecules that are normally catabolized by lysosomal enzymes. Substrate accumulation in the lysosomes causes progressive damage and death to affected cells, tissues, and organs [5, 6]. Clinically, LSDs are a highly diverse collection of diseases that have variable presentations and broad symptom spectra, with patients presenting from the prenatal period through adulthood. Symptoms typically reflect the location and type of substrate accumulation and may include organomegaly, connective-tissue pathology, respiratory and cardiac problems, and central nervous system (CNS) manifestations, such as developmental delay, cognitive regression, behavioral abnormalities, ataxia, and seizures. Most LSDs are life-limiting or life-threatening, and result in substantial morbidity; however, there is considerable variability in the rates of progression of LSDs, often with rapidly progressive disease courses in pediatric patients and slower, more attenuated progression in older patients. This variability results in the need for a variety of outcome measures to assess the clinical course of disease in individual patients. Additionally, although individually rare, collectively, LSDs are estimated to affect as many as 1 in 5,000 births [7, 8]. With the advent of newborn screening for several LSDs, including Fabry disease and Pompe disease, estimates of the incidence of LSDs are improving [9].

Lysosomal storage diseases can be broadly categorized based on the macromolecule that accumulates in cells and tissues. Categories include the sphingolipidoses, mucopolysaccharidoses, mucopolipidoses, oligosaccharidoses, lipidoses, and the glycogen storage diseases. Historically, for most LSDs, treatment has been primarily directed at the general medical management of complications from the disease and

at amelioration of symptoms. More recently, however, ERTs and disease-specific small molecule therapies for some of these diseases have become available. ERTs are intended to replace the deficient enzyme, and to modify or ameliorate disease progression. Current FDA-approved ERTs for lysosomal storage diseases are listed in Table 1.

Although there are no cures for LSDs, ERT has become the principal treatment for many and represents a major advance in their management. For some patients, however, immune responses to ERT have resulted in adverse reactions, including mild to life-threatening hypersensitivity responses [10, 11, 12]. Management strategies for acute hypersensitivity include treatment with antihistamines and antipyretics, and slowing the rate of infusion, and patients can generally be successfully managed without interruption or discontinuation of treatment [13, 14, 15].

More recently, IgG-mediated immune responses, including both neutralizing and non-neutralizing antibodies (Abs), have been shown to affect patients by decreasing the efficacy of ERT as demonstrated by disease progression and losses of previous gains [16, 17]. There has been recent success preventing IgG-mediated immune responses in patients with infantile-onset Pompe disease (IPD) using ITI strategies [18, 19, 20]. However, this strategy has not yet been adopted in other LSDs, where immune responses also occur, but where the clinical impact of such responses on efficacy and safety of ERTs is less clear. Ongoing research aims to determine ways to measure the impact of these antibodies on specific clinical endpoints as well as biomarkers likely to be predictive of clinical outcome.

3. Session 1: Immune Responses to Enzyme Replacement Therapy

A. *The Impact of Anti-Drug Antibody Development on ERT: FDA Experience* -- Amy Rosenberg, MD

Immune responses to therapeutic enzymes may affect both patient safety and clinical efficacy, and clinical effects of most immune responses to therapeutic proteins, including ERT, appear to be mediated by humoral mechanisms. [10-12, 16, 17, 21]. Therapeutic enzymes are taken up by antigen (Ag) presenting cells, either dendritic cells or Ag-specific B cells, which process and present peptides of the ERT to helper T cells specific for the peptide in the context of human leukocyte antigen (HLA) molecules. The role of the helper T cell in generating immune responses to ERT cannot be overstated. Helper T cells signal and activate Ag-specific B cells to proliferate and differentiate into memory B cells, and into Ab secreting plasma cells (both short- and long-lived). The mechanism of Ab response to therapeutic proteins is shown in Figure 1.

Anti-drug antibodies may affect the activity of ERT by several potential mechanisms (Figure 2).

Antibodies that bind to segments of the therapeutic enzyme that are not associated with particular functional activities (i.e., binding, non-neutralizing Abs) bind to the ERT and, when present in high titer, may block ERT activity in the target cell by causing uptake of ERT in Fc receptor (FcR) expressing cells such as monocytes and macrophages. Via this pathway, degradation of the Ab-Ag complex facilitates immune responses. In rare instances, ADAs may act as a chaperone or carrier for therapeutic proteins, potentially enhancing their pharmacokinetics and product activity; however, this type of Ab response is unpredictable and not well understood. Neutralizing Abs are specific for functional domains of the ERT, the uptake domain (uptake neutralizing Abs) which block entry of the enzyme into the cell or into the lysosome through the mannose-6-phosphate (M6P) receptors, or to the catalytic domain (activity neutralizing Abs). Antibodies to the catalytic domain may allow uptake of the ERT into the lysosome, but many factors determine the effects of such Abs on enzyme activity and thus, on product efficacy. First,

development of neutralizing Ab is frequently associated with a high sustained Ab response which may divert Ag-Ab complexes into FcR bearing cells and therefore, for most LSDs, away from critical target tissues. Second, small Ag-Ab complexes may recirculate via FcRn and thereby be denied access to the lysosome. Finally, although the acidic environment of the lysosome would be expected to dissociate enzyme from Ab to the catalytic domain, it is not clear that this is always the case. Dissociation in the context of the lysosome may depend on many factors including Ab affinity, the ability of lysosomes in patients with LSDs to reduce pH to a level to cause dissociation, and additional proteolytic activity which could act on either Ab or enzyme and cause dissociation.

Patients with LSDs have variable residual amounts of the endogenous cellular enzyme affected by their specific mutation. This residual enzyme has been termed cross-reactive immunologic material (CRIM). The level and nature of the residual enzyme affects a patient's propensity to generate ADAs. CRIM can be detected by a western blot in which monoclonal or polyclonal Abs raised to the enzyme are used. Patients with residual endogenous enzymes (CRIM-positive) generally have gene mutations which allow for the production of some cellular enzyme, with or without actual enzyme activity. Exposure of the immune system to the intrinsic enzyme protein (even if non-functional) appears, to some extent, to tolerize the patient's immune system to ERT. For example, CRIM-positive patients with Pompe disease often have low Ab titers, or initial detectable titers, which diminish or disappear over time and generally have a good clinical response to ERT, though this is not always the case [16, 22]. In contrast, CRIM-negative patients with autosomal recessive LSDs typically have 2 null mutations (or in the case of X-linked recessive LSDs, 1 null mutation), and are unable to form any native protein (i.e., they have "knockout" (KO) phenotype). These more severe mutations associated with CRIM negativity may result in more significant immune responses to ERT, because the ERT is recognized as foreign by their immune system, and such patients often exhibit high and persistent Ab titers and neutralizing Abs. Such patients

often have a poor clinical response to ERTs [21, 22]. This experience is highly similar to that of hemophilias A and B in which patients with absent or truncated factors developed Abs and required tolerance induction to reestablish effectiveness of replacement factors [23].

The impact of endogenous enzyme, or CRIM status, on immunogenicity risk has been described in IPD ([22]; discussed in detail in section 4B). In that study, CRIM-negative patients developed higher titer and more sustained Ab response in comparison to CRIM-positive patients. Although this analysis is limited by the small number of patients, the significant differences in clinical outcomes between the two groups and the temporal association with Ab responses suggest that CRIM-negative status in infants with Pompe disease treated with recombinant human acid alpha glucosidase (rhGAA) predicts a poorer clinical outcome, and this reduced response may be mediated by Ab responses to rhGAA. (The much more favorable outcome in CRIM-negative patients who underwent immune tolerance induction is further evidence of the role of Ab responses in determining poor clinical outcome. In CRIM-negative patients who were successfully tolerized, the outcome in terms of invasive ventilation and death was akin to that of CRIM-positive patients.)¹

There has been little published data related to immune response for other LSDs. However, pharmacodynamic data from clinical studies in other LSDs [24], as well as animal model data, suggest that the *in vivo* lessons from IPD may provide insight and make possible recommendations in other LSDs, albeit with a need for further data collection and analysis.

Clinical studies in Mucopolysaccharidosis (MPS) I have shown that ADA to ERT is associated with increased urinary excretion of glycoaminoglycans (GAGs) [21, 25, 26]. Studies in the canine model of MPS I have demonstrated that ADA can alter the distribution and effectiveness of ERT at the tissue level, and that immune tolerizing regimens can prevent ADA formation and improve the effectiveness of ERT as shown by enhanced enzyme activity in critical target tissues as compared to the enzyme activity in such tissues in non-tolerant dogs [27]. Moreover, in murine KO models of Fabry Disease, ERT activity was dramatically decreased in target tissues in seropositive as opposed to seronegative animals. [28].

Our understanding of an individual's risk of generating ADAs has grown, and we are able to measure an individual's Ab response over the course of therapy. The management of Ab responses should be guided by their clinical consequences. When immune responses that can substantially impact efficacy can be predicted, prophylactic immune tolerance protocols should be strongly considered. This is the case with CRIM-negative IPD patients, for example, where the consequences of Ab responses to rhGAA are severe and well recognized. The clinical consequences of ADAs are less clear in other LSDs, such as Fabry disease, where the long-term clinical impact of ADA are not well described or understood [29-32]. This may be because clinical endpoints are not as rapidly reached as they are in Pompe disease. Nonetheless, the preponderance of clinical and animal model data support the negative impact of Ab responses to ERT and the dramatic improvement of such with ITI. The preponderance of data regarding the diminished efficacy of ERT in the setting of a robust immune response in IPD, as well as the experience with prophylactic immune tolerance in these patients, should prompt consideration of ITI for those with LSDs other than Pompe disease at high risk of development of such immune responses.

¹Post-meeting commentary added for purpose of clarification.

B. Anti-Drug Antibodies in Patients with Lysosomal Storage Diseases: Clinician's Perspective --

Barbara Burton, MD

Clinicians have 2 major concerns associated with ADAs in patients with LSDs. First, whether or not ADAs will lead to adverse drug reactions, and second, whether or not ADAs will result in a lack of, or diminished, treatment effect or a negative impact on the outcome of a patient's therapy.

Immune-mediated adverse reactions include immediate hypersensitivity reactions such as anaphylaxis, as well as non-acute type III hypersensitivity responses including immune complex-mediated disease [33]. (The term *infusion-related reaction* (IRR) generally refers to immunologically based adverse events with a temporal relationship to therapeutic protein or drug infusion. This term has been discouraged; however, an agreed-upon definition is lacking. More-descriptive terminology should be used to define the reaction, noting the timing, duration, and specific signs and symptoms observed upon administration of a therapeutic protein product [33]). Hypersensitivity reactions including urticaria, rash, and bronchospasm are commonly observed adverse reactions with ERTs to treat LSDs. Although anaphylaxis has occurred, fortunately it is not common in patients treated with ERTs [12, 13].

Patients with Ab formation are generally more likely to experience hypersensitivity reactions, including anaphylaxis, and patients with these immune-mediated adverse reactions are more likely to have ADA [34]. Table 2 summarizes the estimated rate of infusion reactions and Ab formation with various ERTs used to treat LSDs, based on data derived from clinical trials.

Real-time access to ADA testing is not always available. Clinical management of patients with acute reactions is often guided by treatment algorithms and bedside ADA testing may not alter clinical decision making. Infusion-related reactions with ERT are usually successfully managed by general medical measures such as altering infusion times and schedules and with prophylactic therapies (e.g., treatment with antihistamines and corticosteroids) and acute management of reactions [33]. Determination of the underlying mechanism is of interest, because IgE Ab involvement may have prognostic implications for repeat exposures to ERT and may guide future management (e.g., treatment with IgE mAb) [35]. Although infusion reactions can be challenging to manage in some patients, they should not require discontinuation of lifesaving ERT [36-38]. For patients with more attenuated forms of disease, the benefits of therapy need to be balanced with the risks and burdens of continued treatment when the patient is experiencing IRRs.

Anti-drug antibodies may also affect the efficacy of ERT for LSDs. Although the relationship between ADA and loss of efficacy was well known in factor replacement therapy for hemophilia, it was not widely expected or viewed to be a significant problem in the early days of ERT for LSDs. Early successful treatment of Gaucher disease with ERT suggested that ADA would not be a significant problem in the management of LSDs. Since all patients with type 1 Gaucher disease have residual enzyme activity, they may tolerize their immune system to ERT and result in less risk for developing ADA. (Additionally, since the macrophage is the target cell in Gaucher disease, the presence of non-neutralizing ADA may not lead to mistargeting since uptake by phagocytic cells through their Fc receptor may take place).² In fact, only 15% of patients treated with imiglucerase (Cerezyme[®]) developed ADA. Similarly, fewer than 2% of

patients on velaglucerase (VPRIV[®]) developed ADA, and Gaucher patients generally do well on therapy. (Because the assays used to assess immunogenicity are specific to the ERT, it is not possible to directly compare immunogenicity rates across therapies. However, the preponderance of evidence in Gaucher disease suggests that ADA is not a significant problem in its management.)² The potential negative impact of ADA on treatment effect in LSDs became evident with the treatment of IPD, where despite early intervention, some patients respond poorly or lose response to ERT as a result of immune responses to their therapy [16, 22]. The effect of ADA in other LSDs is more difficult to evaluate than in Pompe disease, due to the slowly progressive natural history of the diseases; however, in most cases, the impact of ADA likely lies somewhere between the Gaucher disease and IPD clinical experiences [21].

A case example to illustrate the impact of ADAs on response to therapy was presented. The patient is a young boy with MPS II, high-titer Ab, and lack of efficacy of his ERT. His presentation was fairly typical of MPS II. He was diagnosed at age 17 months based on coarse facial features, hepatosplenomegaly, and multiple joint restrictions. The diagnosis was confirmed by enzyme assay showing deficient iduronate-2-sulfatase (IDS) activity, and a microarray revealed a complete deletion of the IDS gene, which predicted a severe phenotype. He was started on ERT with idursulfase (Elaprase[®]) shortly after diagnosis, but 2 years after the start of therapy, there was no significant decline in his urinary GAG levels and no evidence of a clinical response. His idursulfase dose was doubled, but there was no change in his clinical condition. The anti-idursulfase IgG Ab titer was 204,000 and these IgG Abs blocked 100% of the idursulfase activity (i.e., 100% neutralizing Abs). In addition, he was positive for anti-idursulfase IgE Ab with a titer of 160, and he experienced some mild IRRs consisting primarily of urticaria and rash. In view of his lack of clinical response, IRRs, and continued Ab production, ITI was initiated when the patient was 5 years of age using a regimen similar to the one developed at Duke (described by Dr. Kishnani, see

section 2. B.), with the exception of the use of ofatumumab instead of rituximab. By the end of the initial treatment period, there was little response in urinary GAG or ADA titers, so the regimen was intensified with an additional course of bortezomib combined with dexamethasone. After 6 weeks on the intensified regimen, a significant decline in the Ab titer along with some decline in the urinary GAG level was observed. A maintenance regimen of monthly ofatumumab, methotrexate, and intravenous immune globulin (IVIG) was initiated. The patient continued to experience hypersensitivity reactions, which worsened to include urticaria, rash, and anaphylaxis, as well as adverse reactions to ofatumumab, for which he was treated. He also experienced additional side effects including hypokalemia, edema, and irritability, attributed either to the drugs or to the pre-medications. Nonetheless, the patient was eventually able to tolerate ERT infusions, and has done well. His ADA titers have declined further (see Figure 3), urinary GAGs have declined, liver size has decreased, and the family has noted an improvement in his walking ability and joint range of motion. He continues to receive his maintenance immune tolerance regimen.

²Post-meeting clarifications

C. Assessing immunogenicity During Clinical Development: Industry Perspective -- Rekha

Abichandani, MD³

Industry recognizes that the assessment of immunogenicity and the clinical relevance of immune response are important components of the safety and efficacy of a medicinal product. Immunogenicity risk assessment begins early in product development and is considered throughout clinical development during the investigational stage and after approval. FDA Guidance for assay development for immunogenicity testing of therapeutic proteins recommends a multi-tiered approach to Ab testing: an

initial highly sensitive screening assay, if positive, is followed by a confirmatory assay [39]. If the confirmatory assay is positive, then an ADA titer is reported and the patient sample is further tested for neutralizing Abs, which are then used to assess the ADAs' ability to neutralize the pharmacological activity of the drug. In the case of ERT products, the neutralizing Ab assay is usually an enzymatic assay to assess the ADA's ability to inhibit the enzymatic activity of the ERT product and/or a cell-based uptake assay to assess the ADA's impact on the cellular uptake efficiency of the ERT product. Similarly, titers are reported if neutralizing activity is detected and confirmed positive. Drug-specific IgE is assessed in the event of suspected hypersensitivity.

Assay development typically begins early for use in Phase 1 trials, and fully validated assays should be in place for pivotal trials. These are usually sensitive and validated assays although assay formats may vary. Enzymatic and uptake or receptor binding neutralizing Ab assays are expected to be in place for pivotal or post-approval trials. The challenge is the time taken to develop validated assays and the difficulty in developing sensitive cellular uptake assays. There has been extensive discussion about CRIM assessment, which has traditionally been evaluated using western blots. However, there is currently no universally accepted methodology for assessment of CRIM status across the spectrum of LSDs. Although CRIM assays are not a statutory requirement at this time, efforts are being made to link the causative genetic mutations in many LSDs to CRIM status. (Efforts are being made to link the causal genetic mutations in many LSDs to CRIM status, where genetic ablation of both alleles (or null mutations) and nonsense mutations imply a possible CRIM negative status. Situations in which there are frame shift or missense mutations that can result in aberrant expression of some protein forms present with less certain CRIM status. Assays to determine residual enzyme function have also been used as surrogate assessments of CRIM.)⁴

Several patient- and product-specific factors need to be considered in the assessment of immunogenicity risk. Patient-specific factors that may be especially important for ERTs include the route of administration, the dosage, frequency and duration, and CRIM status. Product-specific factors for ERTs include host cell protein contaminants and non-human post-translational and chemical modifications (e.g., oxidation, deamidation) and precipitants.

The impact of a patient's genetic status on their immunogenicity risk is being increasingly recognized. CRIM status may provide a simplified assessment of immunogenicity risk, but it should be recognized that CRIM-positive individuals may also develop ADA with significant clinical impact. (Thus, the CRIM assay itself should be optimized to cover the widest coverage of the normal protein. The fate of the endogenous enzyme protein in CRIM positives who develop high titer antibody responses should be evaluated for exposure to the immune system (i.e., the endogenous enzyme protein may be degraded and not processed and presented to the immune system.)⁴ Although the approach to the development of immunoassays and bioassays during clinical development is becoming more consistent, there is little consensus on how to approach the assessment of CRIM status during clinical development. Genotype data should be collected throughout clinical development and evaluated for their correlation to immune responses, even though the impact of genotype on immunogenicity, and its long-term significance, may not yet be well understood.

Attempts should be made to characterize the immune response during drug development and determine its impact on efficacy. However, this approach can be challenging in small patient populations unless dramatic effects are observed. It is particularly challenging to interpret the

significance of ADAs early in clinical development, when biomarkers rather than clinically meaningful outcomes may be informing decision making. (A biomarker is defined as a physiologic, pathologic, or anatomic characteristic or measurement that is thought to relate to some aspect of normal or abnormal biologic function or process. [40-42])⁴ At this stage of development, biomarker correlation with clinical outcome measures may not have been established, and longer-term treatment may be needed to demonstrate clinical benefit. Thus, early development of disease biomarkers, possibly through the use of spontaneously arising or induced animal models of LSD may accelerate development of markers useful in clinical evaluation. It is also important to recognize the need for longer-term follow-up and continued availability of these assays after drug approval, to allow for better understanding of the impact of ADA on clinical outcome.

There are also key considerations from an industry perspective on immune tolerance trials. It is important to identify patients for whom prophylactic immune tolerance may be appropriate. An individualized risk-based approach is needed for each disease and each treatment to assess the risk of an untreated disease or partially effective therapy versus the risk of an immune tolerance regimen itself. There are many immune tolerance regimens under investigation which are not currently approved for the proposed indication or for use in pediatric patients. Consideration should be given to the timing of such trials (i.e., relative to the demonstration of clinical impact of the ADAs and regulatory approval of the ERT).

In summary, from an industry perspective, a comprehensive approach to immunogenicity assessment is a necessary component of modern drug development programs, although assay formats do vary. A risk-benefit assessment, which may need to be individualized to disease, drug and even (mutationally defined) patient populations, should be considered when implementing ITI.

³Disclaimer: Dr. Abichandani is an employee of Shire and is presenting an Industry perspective. This presentation does not represent a formal consolidated position of either BIO or any of the other companies within BIO.

⁴Post-meeting clarifications

4. Session 2: The Role of Immune Tolerance Induction in Enzyme Replacement Therapy

A. Pharmacologic Approaches to Immune Tolerance -- Laurence A. Turka, MD

In the fields of transplantation and auto-immunity, tolerance is not merely the absence of an immune response, but rather an active process of protective immunoregulatory responses. By definition, this process is self-sustaining without a need for ongoing therapeutic intervention. Most immune responses against Ags, not encountered previously, are initiated by T cells. Even most B cell responses leading to Ab production require T cell “help” in the form of membrane bound and soluble signals. It is interesting to note that, with the exception of the response to organ and tissue transplants, the proportion of T cells responding to foreign Ags is remarkably small, approximately 1 in 100,000. Thus, immune responses are directed by a very small number of cells (i.e., Ag-specific T cells) that are able to respond very quickly to an epitope of a foreign protein or a foreign peptide, with a doubling time of approximately 6-8 hours. With these considerations in mind, establishing tolerance to any Ag is a balance between the ability to delete or inactivate Ag-reactive cells and the ability to generate an effective regulatory cellular immune response. If Ag-reactive cells can be curtailed and culled and

conditions established to favor emergence of regulatory cells and activity, tolerance can be induced.

Among the most potent regulatory populations are Foxp3⁺ regulatory T cells, so-called Tregs, which are primary controllers of immune homeostasis.

Approaches to immune tolerance can be divided into three broad categories: Ag-specific, Ag non-specific, and delivery of negative/regulatory signals. Ag-specific therapy delivers Ag in some form that is inherently or relatively tolerogenic. Examples of Ag-specific therapy are the delivery of a peptide or a protein that is coupled to major histocompatibility complex (MHC) molecules, which under varying circumstances may be tolerogenic, and delivery of Ag via specific routes that generally do not promote an inflammatory immune response (e.g., the gut). Antigen non-specific therapy blocks signals necessary to initiate or to sustain an immune response, assuming Ag delivery is occurring endogenously, for example, blocking T cell co-stimulatory signals (such as CD28), which are important for both cell-mediated responses and Ab production, or blocking inflammatory cytokines. Lastly, negative or regulatory signals (e.g., via CTLA-4 or PD-1) might be deliberately delivered to inhibit immune responses. While this is not yet possible with pharmacologic agents, it is likely part of the mechanism of cellular therapy with Tregs.

Lessons learned from tolerance studies in transplantation and autoimmunity may prove instructive in the field of ERT. First, achieving tolerance in small animals does not necessarily translate into success in non-human primates and humans. As a result of lifelong exposure to diverse pathogens, non-human primates and humans have large numbers of memory T cells that, due to antigenic mimicry or the existence of two distinct antigenic receptors on a given T cell, can react to other Ags. These memory

cells are difficult to delete or suppress, and therefore, it is important to consider tolerance inducing treatments before a T cell and Ab response is generated. Based on a review of the literature, a combination of rituximab, methotrexate, and IVIG seems to prevent ADAs in immunologically naïve (i.e., no neutralizing Ab) IPD patients who are receiving ERT. In those with pre-existing Abs, this regimen plus bortezomib seems to be most effective thus far [18].

To conclude, if immune tolerance therapy is considered for ERTs, it is easier to prevent ADA against ERT than it is to suppress an existing Ab response, since memory cells are harder to eliminate than naïve Ag-specific T and B cells. Intervention in patients highly likely to develop high sustained Ab responses during treatment with ERT (e.g., CRIM-negative Pompe disease patients) should occur before or concomitant with the initial exposure to Ag. In general, low-dose continuous Ag is less immunogenic than episodic high-dose therapy. Combination therapies targeting B and T cells might be a possibility, and one should look for what has worked in Ab-mediated autoimmune diseases.

B. Immune Tolerance Induction in ERT to Treat Infantile-Onset Pompe Disease: Current Practice -

- Priya S. Kishnani, MD

Pompe disease is an autosomal recessive metabolic myopathy with a continuum of clinical manifestations, commonly involving cardiac, skeletal, and smooth muscles, due to the deficiency of the lysosomal enzyme acid alpha-glucosidase (GAA). Infantile-onset Pompe disease (IPD) is rapidly progressive, and presents within the first few days to weeks of life. The majority of patients die before the age of 1 year due to cardiorespiratory failure. Since the approval of alglucosidase alfa (rhGAA) in 2006 as the first treatment for Pompe disease, the natural history of this disease has changed [43, 44].

There are now teenagers living with IPD [45]. Although there are challenges with the current ERT, the importance of this lifesaving therapy cannot be overemphasized.

The following topics will be discussed in this section with the focus on the role of immune modulation in Pompe disease: (1) lessons learned from the natural history of IPD patients on ERT monotherapy, (2) immune response observed with early ERT, and (3) experience with ITI to date.

Lessons Learned from the Natural History of IPD Patients on ERT Monotherapy

Although ERT has improved the overall clinical outcomes in IPD, many factors are believed to affect the treatment outcome. Immune responses against ERT resulting in high and sustained anti-rhGAA IgG Ab titers (HSAT) is identified as a poor prognostic factor [16]. Although HSAT is commonly noted in CRIM-negative patients, it can also be seen in a subset of CRIM-positive patients. Patients for which any presence of GAA can be detected on western blot are considered CRIM-positive, and those in which protein is undetectable are considered CRIM-negative, but CRIM status should not be interpreted as an “either/or” phenomenon, but rather a continuum. Hence, CRIM status should be confirmed based on the western blot and GAA mutation analysis, or based on mutation analysis alone if they are known mutations. The ability to predict CRIM status accurately based on underlying genotype is approximately 92%. Since there is a subset of CRIM-positive patients that mount an immune response, clearly other factors in addition to CRIM status influence the clinically observed immune response.

Studies have demonstrated that ERT-treated CRIM-negative patients usually mount a robust immune response against ERT leading to HSAT, whereas CRIM-positive patients generally have low to no Ab titers [16]. A retrospective chart review conducted on 32 IPD patients, stratified based on their CRIM status, revealed that the majority of the CRIM-positive patients (n=21) survived, whereas all the CRIM-negative

patients (n=11) were either deceased or invasively ventilated by the age of 27.1 months [22]. Another retrospective chart review on 34 IPD patients (11 CRIM-negative, 23 CRIM-positive) showed that a subset of CRIM-positive patients (n=9) also developed HSAT with poor clinical outcomes similar to CRIM-negative IPD patients [16]. These data suggest that patients with HSAT respond poorly and experience clinical decline despite ERT. Additional factors, such as the age and the stage of disease at ERT initiation, also contribute to clinical outcome [45, 46]. A recent study that focused only on the treatment outcomes of CRIM-negative patients also suggested that the majority but not all of CRIM-negative patients mounted a significant immune response [47]. The ones that did not develop a significant immune response had unique genotypes, either a frameshift or a splice site mutation in *GAA* [47]. Thus, we should consider CRIM status also as a continuum, from CRIM-negative at one end to strongly CRIM-positive at the other end of the spectrum. Effectively mitigating the immune response to ERT is thus an area of therapeutic intervention where potential improvements can be made to significantly improve clinical outcomes [18, 19, 48, 49]. An important focus in the future is the early identification of patients with Pompe disease who are at greatest risk of developing HSAT subsequent to ERT initiation.

Immune Response Observed with Early ERT

Given the advent of newborn screening for Pompe disease, it is of paramount significance to determine whether early initiation of ERT (<1 month of age) averts the development of an immune response. In order to assess whether early initiation of ERT prevents a robust immune response, patients who started ERT prior to 31 days of age were identified. In one study, 24 of 28 (85.7%) IPD patients who initiated ERT within first 31 days of life seroconverted [50]. The median peak titer for these 24 patients was 6,400. Five of the 24 patients (21%) who seroconverted had peak titers $\geq 25,600$ sustained for periods of time ranging from 3 months to > 1 year. In a second study conducted under a Duke IRB-approved protocol

(PRO00001562), 4 patients (3 CRIM-negative [18] and 1 CRIM-positive [51]) who initiated ERT at age less than one month developed high titers. One patient died at the age of 18 months; 2 patients (1 CRIM-negative and 1 CRIM-positive) were rescued with an ITI protocol that was initiated as they were mounting increasing titers, and the fourth patient (CRIM-negative) had sustained intermediate titers and died at age 45 months. In summary, these results suggest that early ERT treatment does not necessarily prevent an immune response from occurring.

Experience with Immune Tolerance Induction (ITI) to Date

Clinicians typically face 3 primary settings with regard to an immune response to ERT and intervention with immune modulation. The ideal setting for immune modulation is when it is possible to identify the appropriate candidate prior to initiating ERT (*i.e.*, treatment naïve) so that ITI can occur alongside ERT. The second setting is when at-risk patients are identified after an early exposure to ERT and treated prior to development of HSAT. The third setting is when patients are treated after they have developed HSAT, *i.e.*, antibody titers $\geq 51,200$ at two or more occasions at or beyond 6 months on ERT (also known as an entrenched immune response).

In Pompe disease, it is possible to determine CRIM status in approximately 92% of cases based on genotyping, which can be completed in less than 2 days [52]. This has helped greatly and allowed for initiation of ITI without delaying initiation of ERT. In general, approximately 66-68% of IPD patients are CRIM-positive and about 32-34% are CRIM-negative [22, 47]. Overall, the risk-benefit profile favors treating CRIM-negative patients with ITI, because the likelihood of developing HSAT in CRIM-negative cases is extremely high [47]. Currently accepted practice for CRIM-negative patients is to initiate ITI at the time of initiation of ERT and to monitor anti-rhGAA IgG Ab titers monthly [49].

Experience with ITI in the Naïve and Early-ERT Setting

Eighteen CRIM-negative IPD patients from different institutions across the globe have received ITI based on an IRB-approved study at Duke Medical Center. Sixteen of these patients were ERT-naïve when ITI was commenced, and 2 patients had initiated ITI after starting ERT. Data from 11 CRIM-negative patients who received ITI based on the described protocol have been published [19, 49]. Nine patients were ERT-naïve, and 2 had recently commenced ERT. ITI consisted of rituximab and methotrexate, with or without IVIG. The goal of treating patients in the naïve setting is to prevent a cascade of immune response that could result in production of significant and sustained Ab titers upon exposure to ERT. As shown in Figure 4, the treatment-naïve patients were treated with 4 doses of intravenous (IV) rituximab (375 mg/m²; or if body surface area < 0.5 m², 12.5 mg/kg), 9 doses of low-dose methotrexate subcutaneously or orally (0.4 mg/kg) and/or monthly IVIG (400-500 mg/kg) over a 5-week period, along with ERT (alglucosidase alfa 20 mg/kg every other week). IVIG was added as a means of passive immunity because the regimen can deplete B cells, and the target population consists of very sick infants. IVIG is also known to have favorable immunomodulatory properties at higher doses [53]. Depending on their immune response, some patients required an additional cycle of ITI. The decision to repeat ITI was based on collective experience from various experts, and was guided by anti-rhGAA IgG Ab titers and CD19 cell counts, which were used as a marker of B cell recovery [49].

As shown in Figure 5, if patients had no or low Ab titers (defined by the group of experts as a titer of <6,400 in IPD) and showed CD19 recovery at 5 months or later (based on the half-life of rituximab), they continued to be monitored clinically. If patients continued to have an Ab response after B cell recovery, they underwent repeat ITI. Patients were considered immune tolerant if they no longer manifested Abs or they had titers <6400 to the therapeutic protein (and demonstrated CD19 recovery in the face of

exposure to the Ag [ERT in this instance]), and if they are able to mount an immune response to other Ags such as diphtheria or tetanus toxoid vaccines. Most patients underwent ITI in the outpatient setting.

Early studies demonstrated that the expected age of death or age at invasive ventilation of CRIM-negative patients on ERT monotherapy is approximately 27.1 months; however, immune modulation changed the course of the CRIM-negative patients as many are living past 27.1 months and some are even able to be weaned off invasive ventilation (Table 4). The mean age of the cohort of 11 ITI-treated CRIM-negative patients is now 5.7 years (range: 3.7 to 8.7 years); unfortunately, 4 of these patients, aged 1.3 to 4.7 years, died due to disease progression, underscoring the variability even with ERT and successful ITI. However, all were tolerized and the 7 patients who are alive continue to receive ERT. Additionally, all received their routine childhood immunizations and those who received tetanus toxoid and diphtheria vaccinations were shown to mount Ab responses. CD19 cells have also shown recovery (Table 5). Two patients required a second round of ITI due to rising Ab titers (to approximately 6,400) and continue to do well clinically.

Experience with ITI in the Entrenched Immune Response Setting

Duke University Medical Center also has experience with ITI in patients with HSAT, indicative of an entrenched immune response, in both CRIM-positive and CRIM-negative patients. Although rituximab was able to target B cells via their CD20 cell-surface receptor, it became apparent that plasma cells present in the entrenched immune response would not be targeted using a CD20-based regimen, because plasma cells do not express CD20. However, if these plasma cells are not eliminated, they will continue to produce Abs and result in an unfavorable clinical outcome. In this situation, it is important to also target such long-lived plasma cells in addition to B and T cells. Bortezomib (Velcade®), a proteasome inhibitor, was identified as a potential candidate. Bortezomib is approved for the treatment

of multiple myeloma, a plasma cell tumor, and has also been used for kidney transplant rejection with some success [54].

The first patient to be treated with bortezomib was a young boy with CRIM-positive IPD, who was first started on ERT at age 5.4 months [18]. He did well on ERT for 6 months, but started to show clinical decline, including loss of motor function and need for bilevel positive airway pressure (BiPAP) ventilation at about age 14 months, with very high Ab titers of 102,400, and rising left ventricular mass index (LVMI). He was treated initially with cyclophosphamide and rituximab, but without improvement. Bortezomib was then added, at a dose of 1.3 mg/m² (see reference 18 for dosing details), in addition to the rituximab, methotrexate, and IVIG regimen (cyclophosphamide was stopped). There was a rapid decline in his titers (from 1:204,800 to 1:100) and an improving LVMI shortly after completion of his first cycle of 4 doses (day 1, 4, 7 and 11) with bortezomib. The patient gained back some upper and lower extremity movement, had a dramatic decrease in LVMI, and a reduction in his ventilator settings [18].

The bortezomib, rituximab, methotrexate and IVIG regimen was subsequently tried in additional patients, including a CRIM-negative patient and a CRIM positive patient with very high Ab titers (819,200 and 204,800 respectively). The 3 patients with previous entrenched responses as published [55] were doing well at the time of this report and range in age from 7.6 to 10.2 years (age calculated on 6/7/2015). No serious adverse events related to ITI have been reported. These results show that it is important to target the T cells, B cells, as well as the plasma cells in entrenched immune response.

In summary, the clinical course of CRIM-negative patients on ERT monotherapy shows that these patients, with very few exceptions, mount a significant immune response with poor clinical outcome, and early ERT treatment (*i.e.*, ERT initiated prior to age 1 month) does not seem to protect against

development of HSAT. CRIM status can be determined early by mutation analysis alone for known mutations, or by western blot plus mutation analysis where CRIM status cannot be predicted by underlying genotype. Immune modulation in the CRIM-negative, naïve setting has shown success with good safety and efficacy in IPD. Patients with high, sustained titers can potentially be tolerized with ERT and immune modulation, including a plasma cell targeting treatment, but this requires prolonged immune suppression and is a significantly higher risk regimen. The risk-benefit ratio in infants with Pompe disease who are CRIM-negative favors the use of immune modulation therapy in the naïve setting. Further work is needed to identify at-risk CRIM-positive patients as well as more attenuated regimens, including Ag-specific tolerance strategies.

A. *Non-Immunosuppressing Approach to Tolerance Induction During ERT Treatment* – Jeanine Jarnes Utz, PharmD

Neutralizing anti-ERT Abs have been shown to hinder the efficacy of ERT by several mechanisms. These Abs can cause reduction in enzyme stability, enzyme degradation in the blood stream, Ab-mediated blockade of cell receptor uptake of ERT, retargeting of M6P-glycosylated enzyme to macrophages, and intracellular misrouting of ERT [56, 57].

Although many of these products have been effective as immune tolerizing agents, the long-term safety of these agents is potentially concerning, especially regarding immune suppression. Table 3 summarizes examples of pharmaceutical agents that are commonly used for immune tolerization in terms of their proposed mechanisms in immune tolerance and potential adverse events.

Patients with MPS diseases suffer from recurrent respiratory and ear infections, secondary to accumulation of mucopolysaccharides in respiratory tissues. In select patients with lysosomal diseases in whom immunosuppression poses a significant safety concern and where achievement of immune tolerance over a longer period of time (e.g., 1-2 years) is acceptable clinically, using an immune tolerance regimen that is less immunosuppressive may be a reasonable option to consider. In patients with severe hemophilia A who have high levels of inhibitors to exogenously administered factor VIII, immune tolerance regimens that use increased exposure to the Ag (i.e., more frequent administration of factor VIII) are often used. Intravenous immune globulin is sometimes included in these regimens as an additional immune modulating agent. Such immune tolerance regimens for patients with inhibitors to factor VIII are reported to have success rates of ranging 51-70% and have the advantage of being non-immunosuppressive [58, 59].

A case study illustrating a successful application of a non-immunosuppressive regimen is that of another Hunter syndrome (MPS II) patient, diagnosed at 14 months of age, began losing clinical response after 3½ years of ERT. He was infection-free for longer periods of time after initiating ERT, but the frequency of respiratory tract infections began increasing again, and he began to have chronic recurrent infections. His dysmorphic features intensified, and his urinary GAG levels increased from 2-3 times the upper limit of normal to 6 times the upper limit of normal. A neutralizing Ab assay ultimately revealed 100% inhibition of idursulfase activity; however, the assay result was not available soon enough to inform clinical care. Because this patient was experiencing chronic infections and had valvular disease from Hunter syndrome that could place him at an increased risk of bacterial colonization of the heart valves under T cell suppression, a non-cytotoxic target regimen was initiated, consisting of more frequent administration of ERT and IVIG [58]. The regimen was adapted from immune tolerance regimens used in

patients with hemophilia who developed inhibitors to factor VIII. The patient received low-dose ERT twice a day at home at a total weekly dose of 1 mg/kg/week (twice the recommended dosage). He also received IVIG (200 mg/kg), weekly. This non-cytotoxic target regimen resulted in reduction of neutralizing anti-idursulfase Abs from 100% inhibition of idursulfase activity to negligible inhibition over 2 years (Figure 7). There was a temporary increase in percent inhibition after the patient's ERT regimen was changed from twice a day to once a day, but the percent inhibition began declining again with continued once-a-day dosing. In addition, his urinary GAG decreased from 9 times the upper limit of normal immediately prior to initiating the non-cytotoxic regimen to the normal range.

The non-cytotoxic regimen used to treat this patient involves a greater daily treatment burden on the patient and his/her family, as well as risks associated with long-term and repetitive IV or port-a-cath access, and may not be appropriate in all circumstances. In addition, it is unclear whether the daily dosing ERT regimen can be successfully reduced to the previously prescribed weekly dosing schedule without a resultant increase in ADA to previous levels.

In summary, the non-immunosuppressive regimen described here may be considered as an appropriate initial option for patients in whom underlying disease may result in higher incidence of infection risk, or in whom achieving immune tolerance over a longer period (1-2 years) is clinically acceptable.

C. Experience with ERT: Two Parents' Perspectives

(i) Perspective - Steve Holland

Mr. Holland shared his experience as the father of three children with the attenuated form of MPS I or Hurler-Scheie syndrome who have received ERT. He relayed that all three of his children were diagnosed with MPS I around the same time. His children had experienced frequent infections, including recurrent otitis media requiring multiple sets of tympanostomy tubes, and none of them could raise their arms above their head, but the diagnosis was only made after one of his children presented with anisocoria.

In 1998, his oldest child participated in the phase 1/2 trial for laronidase. Upon initiation of ERT, he began having more energy, no longer required naps during the day, and demonstrated better range of motion. He experienced side effects from ERT, including gastrointestinal hypersensitivity reactions, presenting with severe abdominal pain, and flushing and wheezing during some infusions, but responded well to corticosteroids, antihistamine and lengthening of infusion duration, and he was able to continue ERT despite intermittent hypersensitivity reactions.

His younger two children participated in the phase 3 trial for laronidase. The family initially traveled weekly to North Carolina and eventually moved there for the rest of the phase 3 trial. His two younger children required wheelchair use in the airport prior to initiating ERT, but one of them was able to push the other one's wheelchair by the second week of ERT treatment, and it became clear to the parents that one was receiving the active drug and the other was receiving placebo, which turned out to be the case when the study treatment assignments were unblinded. Upon approval of laronidase in 2003, all of his children were able to receive laronidase infusions locally and transition to home infusions.

Mr. Holland concluded his presentation by sharing with the audience that his risk tolerance in the hope of potential benefit is greater than the general public. His knowledge that his children's disease is degenerative and terminal gives him the courage to accept the potential risks of intervention. He reflected on the potential alternate outcome: if 10 families who participated in the phase 1/2 trial for laronidase had instead chosen not to accept that risk, or had not been given the opportunity to participate in the trial, then thousands of children would not have had the opportunity to benefit from ERT. This increased risk tolerance is also evidenced by the vast majority of the severe Hurler families accepting significant risks with bone marrow transplantation to stabilize their children's cognition. Mr. Holland additionally stated that he believes ERT has stabilized his children's disease, given them a better quality of life than they otherwise would have had, and that he believes that it is important to get ERT to every patient who needs it, including those with allergic reactions and elevated Ab titers.

(ii) **Perspective - Melissa Hogan**

Ms. Hogan read a statement from another parent who has a 6½ year old child with Hunter syndrome who underwent ITI. This child has MPS II with a full gene deletion and would be predicted to be CRIM negative. He started ERT after being diagnosed at approximately 18 months of age. Although initially he responded positively to treatment with ERT, the effect waned over time. At approximately 4 years of age, this child began developing signs and symptoms of hypersensitivity reactions. Although premedication with antihistamine and a slower rate of ERT infusion controlled his hypersensitivity reactions, his underlying disease worsened. He presented with abdominal distension, increased lethargy, and shortness of breath, and urinary GAG levels were very high despite receiving ERT for more than 2 years. Antibody testing showed that he was both IgG and IgE positive, and his Ab titers have increased steadily. The boy's mother became convinced of her son's need for ITI in order to restore the

effectiveness of ERT, but initially, it was difficult to find a physician who felt comfortable prescribing ITI to her child. Once he initiated ITI, his Ab titers eventually normalized.

Ms. Hogan concluded by sharing a perspective from the Hunter syndrome community on ERT and immune tolerance. She asserted that what is known about the relationship between genotype, ERT response, Ab development, adverse reactions, and the role of ITI needs to be communicated publicly in a way that is easily accessible to patients, caregivers, and local physicians who are prescribing ERT. In addition, she recommended that LSD patients/caregivers should be made aware of their or their child's CRIM status prior to ERT initiation because of the greater challenges and risks associated with normalizing Abs once a patient develops high titers. She also recommended that the implications of CRIM status should be explained to patients/caregivers, and in CRIM-negative patients, ITI should be recommended. She noted that currently, the ERT manufacturers are often the only source of Ab assays and results are only available many months after testing, diminishing their utility; hence, the results of Ab testing should be available shortly after testing to help guide therapeutic decisions. She recommended that FDA require development of prompt and efficient Ab testing following FDA approval of any new or current ERT and to offer such testing in a timely manner for approved ERT products.

5. Discussion Summary and Key Points from the Meeting

Discussion panels and a question and answer session followed each of the two sessions. A summary of the discussion and the key points are as follows:

A. Identifying Patients for Treatment with Immune Tolerizing Regimens

Presenters and panelists noted that most of the data regarding immune responses and ADA levels are from clinical trials with relatively small number of patients and limited periods of treatment and follow-up despite the chronic nature of the diseases. Although some information from clinical treatment experience of immune tolerance regimens in case studies and small patient cohorts is available, it is largely limited to patients with severe enzyme deficiencies or rapidly progressive phenotypes. Data from the use of immune tolerizing regimens in more attenuated forms of these diseases are generally lacking; however, several panelists and speakers noted that chronic immune stimulation and a pro-inflammatory response should generally be regarded as unfavorable for patients and should generally be prevented or treated if possible. Currently, however, in the majority of patients with attenuated forms of disease, there is little data available to inform a benefit-risk determination for immune modulating therapies vs. the chronic, often slow progression of the disease. It was additionally noted that patients studied in clinical trials may not reflect the overall patient population or the potential for immunogenicity. For example, extrapolating results from patients participating in clinical trials with attenuated phenotypes and residual endogenous enzyme to CRIM-negative patients, and vice versa, may not provide the complete picture for a specific product in an individual disease, but these gaps from these studies point toward areas for future research.

Although the data are limited, the case report examples in severe enzyme deficiency patients are compelling. Dramatic responses to immune tolerizing regimens have been observed in a small number of patients, especially in CRIM-negative IPD patients, and individuals with Hunter syndrome. Regimens

recently developed and profiled in case reports and small cohorts generally target B cells and T cells, but these treatment regimens are still evolving in terms of the combination of drugs used, dose, length, and sequence of these treatment regimens are still evolving. Additional analyses of the various regimens, as well as information on how to assess response and modify these regimens to optimize individual effects, are needed. Intervention with prophylactic immune tolerance therapy is likely to be easier, requiring shorter duration of therapy (e.g., currently in IPD, prophylactic therapy duration is approximately 5 weeks), than attempting to intervene once an immune response has been established; however, these are non-Ag specific tolerizing regimens, and the panel acknowledged that while there is consider clinical safety experience with these agents (mainly in adults), there are also associated risks from B cell suppression. Treating an established immune response requires longer-term regimens and possibly higher doses that may increase safety risks in the near and longer term. The panel noted that some patients with severe enzyme deficiencies also present with an established Ab response, which may preclude prophylactic therapy.

It was also emphasized that some of these diseases carry high risks of infection due to the underlying disease (e.g., respiratory infections in Pompe disease, otitis media and respiratory infections in MPS), and that optimizing therapy through immune tolerance regimens may actually lessen infectious complications. Dr. Kishnani related that in her experience in a small number of IPD patients who are at high risk of infection due to severe muscle weakness, no notable increase in risk of infections was seen. Similarly, Dr. Utz's experience in one patient with a non-cytotoxic regimen also showed that the number of infections in this patient decreased with ITI. It was additionally noted, however, that there is little experience in children, especially very young children, on the long-term effects of immune modulating therapies over time. Most experience with immune modulating drugs comes from the treatment of adult patients with various forms of cancer (e.g., chronic lymphocytic leukemia) or autoimmune

disorders (e.g., rheumatoid arthritis) where patients are often treated for long periods of time, with higher exposures than are being proposed for ITI for ERT. Age at onset of treatment may also make a difference, e.g., IPD disease neonates vs. 2-4 year olds with MPS II, who are at different stages of immune system development and maturation.

There was a general consensus among the panel members that for severely affected patients (i.e., CRIM negative patients, patients with absent or severely truncated enzyme proteins) who may be at higher risk of developing ADA and may have deleterious clinical consequences, the following should be considered:

- Prophylactic immune tolerizing regimens should be considered based on a benefit-risk assessment prior to or concurrent with ERT. For example, the benefit-risk assessment should consider the risk of ineffective or attenuated response to ERT due to ADA vs. that of the ITI regimen.
- Immune tolerization in IPD patients appears to have a favorable benefit-risk profile, with few reported side-effects directly related to the regimens. However, it was acknowledged that the safety experience to date with bortezomib (Velcade®) and rituximab (Rituxan®) was mainly in oncology patients or rheumatology patients (rituximab), and the current prescribing information for both drugs lack information for use in pediatric patients. This makes it difficult to extrapolate long-term safety data due to differences in underlying disease. It would be important to carefully collect long-term safety information of ITI to further characterize the safety in the ERT-treated population.
- Long-term assessments of the impact of immune tolerizing regimens on the developing immune system (e.g., long-lived plasma cells that may be needed throughout life) are needed.

- ADA monitoring during therapy in patients with severe deficiencies is essential. Neutralizing Ab monitoring, where feasible, should also be explored; however, the limitations of current assay technologies were also noted.
- There is a need for rapid genetic testing and a better understanding of high-risk mutations in order to appropriately identify patients for prophylactic intervention before or concurrent with ERT initiation.
- The role of industry (manufacturers) and independent laboratories in developing ADA assays and working collaboratively to develop a means of comparing the ADA results from various ERT products is essential. Industry assays are usually product specific, making comparison difficult when there is more than one product on the market for the same indication. Thus, assay development by independent laboratories should be considered and may be preferable.

The role of ITI in patients with less severe enzyme deficiencies and more attenuated forms of the disease is less clear. Although workshop participants were generally supportive of approaches to optimize the therapy for all patients, and the evidence from animal models suggests that ADAs can interfere with or attenuate responses to ERTs, further research is warranted to elucidate the effects of immune tolerizing regimens in patients with attenuated forms of disease. Participants discussed whether to intervene with prophylactic immune tolerizing regimens in all patients with attenuated forms or to treat only if an Ab response develops in individual patients, but there are no data to support a position at this time, and defining a benefit-risk in these patients is not possible with currently available evidence. It was noted that the lack of obvious correlation between ADAs and clinical outcomes in clinical trials of more slowly progressive LSDs should not lead to the conclusion that clinical outcome is unaffected by ADA. There is accumulating evidence that storage materials rise again after

initial fall when ADA occurs (as illustrated by the 2 Hunter syndrome patient case histories presented earlier); however, clinical manifestations may take years to progress, particularly in attenuated forms of disease, and more research is needed to develop good biomarkers, and short-term or sensitive clinical end points, which may allow earlier assessment of ADA's clinical impact. Long-term data are clearly needed to better assess the impact of ADA on clinical outcomes in these diseases. There was much discussion and general support for consideration of randomization to treatment arms with and without immune tolerizing regimens in clinical trials for ERTs; however, small patient numbers and difficulties with powering trials and in interpreting the results by smaller subgroups were noted. Although it was acknowledged that every effort should be made to optimize therapy and remove Ab or immune system interference when delivering a therapy, assessing a clinical response in attenuated forms is especially difficult. More clinical data examining the relationship between results of Ab testing to clinical outcomes are needed before clinical consensus can be reached. It was additionally noted that Ab effects may be long-term, and may not be possible to adequately assess in a premarket clinical trial. Continuous learning in the post-marketing period, for example, through registries or other long-term follow-up mechanisms was discussed. Development of standards, consistent approaches or guidelines to Ab testing, follow-up, and intervention should be considered.

It was noted by some participants that for some products and diseases, attempts to assess the impact of antibodies on clinical outcomes or pharmacodynamic assessments (such as a biomarkers) have shown no evidence of an effect on treatment. Development of comprehensive mutational databases to better predict Ab response could be helpful, although for mutations other than severe deletions or truncations, the current ability to predict Ab response is limited.

Additionally, it was noted that the clinical manifestations and treatment paradigms for the individual diseases are different. For example, under the current treatment guidelines, MPS IH (Hurler disease phenotype) patients are all considered for hematopoietic stem cell transplantation (HSCT), and may receive ERT as a bridge prior to transplant. For these patients, HSCT should be factored into discussions regarding immune tolerizing regimens. Exploration of immune tolerizing regimens for different modes of administration is also needed. For example, although most patients receive IV administration of ERT, intrathecal (IT) administration is under investigation in some diseases. In some clinical trials of IT ERT, the patients were not treatment naïve and had been receiving IV ERT therapy previously. The impact of Ab formation in the CNS is largely unknown. There are potential risks associated with serum and CNS Abs, as well as the potential for infection in IT administration, which are important concerns and may alter the benefit-risk assessment.

Finally, additional research is also needed in the area of IgE-mediated reactions. Their incidence is low, but the potential adverse effects are considerable. It was noted that some centers pretreat with antihistamines and corticosteroids, which also carry risks, and may factor into immune tolerizing treatment decisions.

B. Outcome Assessments and Response indicators

An important theme underlying the ability to assess the effects of immune tolerizing regimens was the current lack of sensitive outcome measures—either clinical outcomes or biomarkers—to guide decision making and to assess the effects of these interventions. Most enzyme deficiency diseases are chronic progressive diseases with high inter-patient heterogeneity in clinical manifestations and rates of progression. Except for a small number of patients with severe enzyme deficiencies (e.g., IPD), distinguishing the effects of elevated Ab titers from the underlying course of the disease may be difficult. Workshop participants urged increased research into biomarkers and clinical pharmacology tools to guide decision making. The panel also supported the use of all available evidence, e.g., from animal models and related diseases (while acknowledging that animal models and related diseases do not fully recapitulate the individual disorders, and animal immune/Ab responses are not predictive of human responses for all diseases and therapies). Thus, this information should be interpreted with considerable caution. Distinguishing between known complications of the disease and inherent natural disease progression vs. inadequate response to therapy is difficult and made more so by the relatively small study population size that can be achieved in rare disease clinical trials. It was also noted that having the ability to distinguish between clinical manifestations that are reversible and those that are irreversible due to disease end stage would be of benefit.

Much of the clinical trials experience with approved ERTs has relied upon outcome assessments that measure higher or integrative levels of function, such as the 6-minute walk test (6MWT) in non-infantile Pompe disease and the MPS diseases. Further development of biomarkers as more rapid indicators of potential pharmacologic effect of ERTs could be useful for monitoring immune tolerizing regimens, but most diseases do not have biomarkers or clinical outcome assessment tools that are sensitive enough or that accurately reflect target organ effects, and better response indicators are needed for many of these

diseases. For example, urinary GAGs are often assessed in the MPS diseases, but they are suboptimal, because they give no information regarding ERT effects at the critical tissue-organ level, and are not indicators of CNS disease.

C. Antibody Testing and Laboratory Methods

Several participants from the academic, treating, and patient communities noted that differences in ADA testing methods, schedules, and reporting processes for the different ERTs limit the utility and interpretability of the results in the clinical setting. There was general consensus that there is a need for better communication, training, and education of healthcare providers regarding the scientific basis and the interpretation of ADA levels. Training should include the entire healthcare team in addition to specialists, including the local physicians (who are likely to order these tests), consultants, and allied health professionals, because patient care is usually multi-disciplinary, and decision making usually involves input from the entire patient care team.

Healthcare providers described additional factors in ADA testing that may further complicate patient care. Most ADA titers are non-standard laboratory tests conducted by specialty labs or drug sponsors, and are not usually offered by commercial labs. This may lead to different reporting procedures, test accessibility, interpretation of values and changes in titers over time, and comparability between testing for individual products. Because much of the ADA testing has been in clinical trials conducted for research purposes and has not been operationalized for clinical practice, turn-around times are very

long, additionally limiting their utility. In clinical use, because there are small numbers of patients needing the assays, lab tests tend to be run in batches, also resulting in slow turn-around times.

Workshop participants from multiple disciplines noted that the ADA results are not translatable across diseases, ERTs, or labs. Assays are product specific, and developed and validated by different sponsors using different methodologies, making comparisons across products ineffectual.

Some key points and research questions from the discussion included:

- In the setting of clinical decline of a patient on ERT, an important purpose of measuring ADA titers is to assess whether the deterioration may be caused by neutralizing Abs. However, Ab titers are usually relatively blunt measures that measure a polyclonal response, and specifically identifying the sites of enzyme activity neutralization can be difficult.
- There was general consensus that topic areas that could greatly benefit from additional research included the following: the specific Ab populations (isotypes) involved in the immune response, finer epitope mapping, assessment of secondary effects (e.g., IL-6), and evolution of the immune response over time with continued ERT exposure and in different age groups.
- The results of ADA assays are dependent on the timing and frequency of blood sampling, in addition to the sensitivity and specificity of the methods used to determine presence of ADAs. Many participants were in favor of developing standardized testing regimens, and exploring more standardized assay development, although it was recognized that given the intricacies of assay development and different assay formats, the latter would be difficult to achieve.

- Better understanding of how Ab responses are mounted to proteins and the nature of the protein responsible (e.g., glycosylation, protein quality, contaminants) are needed and should be a continued area of research.

D. Patient and Caregiver Perspectives

Several speakers from the patient community emphasized that patients and families affected by LSDs are often intimately involved in their or their loved one's medical care. As such, LSD patients/caregivers in general welcome more information, and the need to put Ab levels and immune responses in context based on available data was recognized as an important area for ongoing communication. Additionally, the importance of long-term relationships and involvement of the entire healthcare team, as well as the involvement of local physicians in addition to specialists was re-emphasized.

E. Communication Strategies

The discussion panel noted that more clarity around naming conventions (e.g., what constitutes "CRIM-negative" status), identification of high-risk patients, and dissemination of clinical practices and research in the area of immune tolerance is needed. Establishment of working groups, and collaboration between allied fields, such as transplant medicine, vaccine biology, allergy and immunology specialists, and other disease areas with longer experience (e.g., hemophilia) should be considered. It was noted that a hindrance to achieving these goals is general under-representation of rare disease-related

research and clinical experience in traditional publication venues, such as peer-reviewed scientific journals. This may occur because some journals may be reluctant to publish articles regarding diseases affecting small numbers of patients. A general lack of funding for niche areas of clinical research, particularly for rare diseases was also noted. Proposals for exploration of non-traditional pathways for information sharing and dissemination included the following:

- Patient and advocacy group and industry support for infrastructure building that could facilitate research and data access for research investigators for areas of common interest should be explored.
- Given the small numbers of patients, it would be beneficial for the broader stakeholder community to collaborate on facilitating access to data to address research questions. Issues surrounding proprietary and commercial confidential information, privacy concerns, and publication rights need to be further explored.

6. Concluding Remarks

Enzyme replacement therapies have been available for some enzyme deficiency disorders for several decades and represent an active and growing area of clinical research, drug development and drug approvals. New areas of research into these diseases have been opened with longer-term survival of patients, and the consequences of long-term protein administration are increasingly being recognized, especially immune-mediated adverse events, and are important areas for continued study. Although ERTs have provided considerable benefits to patients for some diseases, we recognize that there is much

work left to be done. At the current time, most patients with enzyme deficiency diseases have no available therapies, and those who do usually have only one option for treatment. Importantly, for areas with available treatments, there is a need to identify barriers to successful treatment and to optimize utilization of available therapy to the greatest possible extent. Given the small populations of patients with the individual diseases, there is a strong need for collaboration across centers, disciplines and companies developing these products, and for the establishment of an infrastructure that supports additional research and maximizes all opportunities for learning from experience.

Acknowledgments

We are indebted to the National Organization for Rare Disorders for their generous sponsorship of this workshop, and to the many individuals and organizations who generously donated their time and expertise to make this event a success. We also wish to thank Drs. Julie Beitz and ShaAvhrée Buckman-Garner of FDA CDER for their careful reading of this manuscript and for their helpful comments.

Disclosures

The following authors declared a financial relationship with these companies and organizations:

R Abichandani: Employee of Shire

J Bluestone: Consulting fees, honoraria and/or research funding from Juno Therapeutics, MacroGenics, Becton-Dickinson Biosciences, Flexus Biosciences, and Pfizer.

BK Burton: Consulting fees, honoraria and/or research funding from Biomarin, Shire, Synageva, Ultragenyx, Genzyme and Hyperion.

PI Dickson: Consulting fees, honoraria and/or research funding from BioMarin, Genzyme, Shire, Isis and Armagen Pharmaceuticals

M Hogan: Speaking fees and expenses in connection with a clinical trial from Shire plc and holds stock in Amicus Therapeutics.

PS Kishnani: Consulting fees, honoraria and/or research funding from Genzyme Corporation; Amicus Therapeutics; Shire Pharmaceuticals; Lysosomal Disease Network

P Tanpaiboon: Honoraria and/or research funding from BioMarin and Genzyme

L Turka: Consulting fees, honoraria and/or research funding from Novartis, Myriad Genetics, NeXption, Pelican Therapeutics, Ipsum

J Utz: Consulting fees, honoraria and/or research funding from Genzyme, Actelion, and Pfizer.

CB Whitley: Consulting fees, honoraria and/or research funding from Actelion Pharmaceuticals, Ltd.; BioMarin Pharmaceutical Inc.; Genzyme Corporation; Pfizer Inc.; Shire; Synageva; Ultragenyx

These authors indicated they have no financial relationship relevant to this article to disclose: M Dewey, A Freitas, D Gavin, D Griebel, S Holland, ZB Kazi, JJ Lee, L Muldowney, AR Pariser, A Rosenberg, YM Wang

References

1. FDA. Immune responses to enzymes replacement therapies: Role of immune tolerance induction. Accessed July 30, 2015: <http://www.fda.gov/drugs/newsevents/ucm392641.htm>.
2. Te Vruchte D, Wallom KL, Platt FM. Measuring relative lysosomal volume for monitoring lysosomal storage diseases. *Methods Cell Biol.* 2015;126:331-47. doi: 10.1016/bs.mcb.2014.10.027
3. Ratko RA, Marbella A, Godfrey S, Aronson N. Enzyme-replacement therapies for lysosomal storage diseases, Technical Briefs, No. 12, Agency for Healthcare Research and Quality (US), 2013, January. Accessed July 30, 2015: <http://www.ncbi.nlm.nih.gov/books/NBK117219/pdf/TOC.pdf>.
4. Schultz M, Tecedor L, Chang M, Davidson B. Clarifying lysosomal storage diseases. *Trends in Neurosciences* 2011;34(8): 401-410.
5. Ballabio A, Gieselmann V. Lysosomal disorders: from storage to cellular damage. *Biochim Biophys Acta.* 2009;1793(4):684-96. doi: 10.1016/j.bbamcr.2008.12.001.
6. Vitner EB, Platt FM, Futerman AH. Common and uncommon pathogenic cascades in lysosomal storage disease. *J Biol Chem.* 2010;285(27):20423-7. doi: 10.1074/jbc.R110.134452.
7. Meikle PJ, Hopwood JJ, Clague AE, Carey WF. Prevalence of lysosomal storage disorders. *JAMA.* 1999;281(3):249-54. doi:10.1001/jama.281.3.249.
8. Meikle PJ, Grasby DJ, Dean CJ, et al. Newborn screening for lysosomal storage disorders. *Mol Genet Metab.* 2006;88(4):307-14. doi:10.1016/j.ymgme.2006.02.013

9. Matern D, Oglesbee D, Tortorelli S. Newborn screening for lysosomal storage disorders and other neuronopathic conditions. *Dev Disabil Res Rev.* 2013;17(3):247-53. doi: 10.1002/ddrr.1117.
10. Kim J, Park MR, Kim DS, et al. IgE-mediated anaphylaxis and allergic reactions to idursulfase in patients with Hunter syndrome. *Allergy.* 2013;68(6):796-802. doi: 10.1111/all.12155.
11. Ponce E, Moskovitz J, Grabowski G. Enzyme therapy in Gaucher disease type 1: effect of neutralizing antibodies to acid beta-glucosidase. *Blood.* 1997;90(1):43-8.
12. Brooks DA, Kakavanos R, Hopwood JJ. Significance of immune response to enzyme-replacement therapy for patients with a lysosomal storage disorder. *Trends Mol Med.* 2003;9(10):450-3. doi:10.1016/j.molmed.2003.08.004
13. Burton BK, Whiteman DA, HOS Investigators. Incidence and timing of infusion-related reactions in patients with Mucopolysaccharidosis type II (Hunter syndrome) on idursulfase therapy in the real-world setting: a perspective from the Hunter Outcome Survey (HOS). *Mol Genet Metab.* 2011;103(2):113-20. doi: 10.1016/j.ymgme.2011.02.018.
14. Miebach E. Management of infusion-related reactions to enzyme replacement therapy in a cohort of patients with Mucopolysaccharidosis disorders. *Int J Clin Pharmacol Ther.* 2009;47 Suppl 1:S100-6.
15. Kim KH, Decker C, Burton BK. Successful management of difficult infusion-associated reactions in a young patient with Mucopolysaccharidosis type VI receiving recombinant human arylsulfatase B (galsulfase [Naglazyme]). *Pediatrics.* 2008;121(3):3714-7. doi: 10.1542/peds.2007-0665.

16. Banugaria SG, Prater SN, Ng YK, et al. The impact of antibodies on clinical outcomes in diseases treated with therapeutic protein: lessons learned from infantile Pompe disease. *Genet Med.* 2011;13:729-736. doi: 10.1097/GIM.0b013e3182174703.
17. Patel TT, Banugaria SG, Case LE, et al. The impact of antibodies in late-onset Pompe disease: a case series and literature review. *Mol Genet Metab.* 2012;106(3):301-9. doi: 10.1016/j.ymgme.2012.04.027.
18. Banugaria SG, Prater SN, McGann JK, et al. Bortezomib in the rapid reduction of high sustained antibody titers in disorders treated with therapeutic protein: lesson learned from Pompe disease. *Genet Med.* 2013;15(2):123-31. doi: 10.1038/gim.2012.110.
19. Messinger YH, Mendelsohn NH, Rhead W, et al. Successful immune tolerance induction to enzyme replacement therapy in CRIM-negative infantile Pompe disease. *Genet Med.* 2012;14(1):135-42. doi: 10.1038/gim.2011.4.
20. Mendelsohn NJ, Messinger YH, Rosenberg AS, Kishnani PS. Elimination of antibodies to recombinant enzyme in Pompe's disease. *N Engl J Med.* 2009;360(2):194-5. doi: 10.1056/NEJMc0806809.
21. Wraith JE, Beck M, Lane R, et al. Enzyme replacement therapy in patients who have Mucopolysaccharidosis I and are younger than 5 years: results of a multinational study of recombinant human alpha-L-iduronidase (Iaronidase). *Pediatrics.* 2007;120(1):e37-46. doi: 10.1542/peds.2006-2156
22. Kishnani PS, Goldenberg PC, DeArme SL, Heller J, Benjamin D, Yong S, Bali D, Smith SA, Li JS, Mandel H, Koeberl D, Rosenberg A, Chen YT, Cross-reactive immunologic material status affects

- treatment outcomes in Pompe disease infants, *Mol. Genet. Metab.* 2010;9:26-33. doi: 10.1016/j.ymgme.2009.08.003.
23. Oldenburg J, Pavlova A. Genetic risk factors for inhibitors to factors VIII and IX. *Haemophilia.* 2006;12 Suppl 6:15-22. DOI: 10.1111/j.1365-2516.2006.01361.x
24. Langereis EF, van den Berg IET, Halley DJJ, et al. Considering Fabry, but diagnosing MPS I: Difficulties in the diagnostic process. *JIMD Rep.* 2013;9:117-120. Doi: 10.1007/8904 2012 189.
25. Clarke LA, Wraith JE, Beck M, et al. Long-term efficacy and safety of laronidase in the treatment of Mucopolysaccharidosis I. *Pediatrics.* 2009;123(1):229-40. doi: 10.1542/peds.2007-3847.
26. Giugliani R, Rojas VM, Martins AM, et al. A dose-optimization trial of laronidase (Aldurazyme) in patients with mucopolysaccharidosis I. *Mol Genet Metab.* 2009;96(1):13-9. doi: 10.1016/j.ymgme.2008.10.009.
27. Dickson P, Peinovich M, McEntee M, et al. Immune tolerance improves the efficacy of enzyme replacement therapy in canine Mucopolysaccharidosis I. *J Clin Invest.* 2008;118(8):2868-76. doi: 10.1172/JCI34676.
28. Ohashi T, Lizuka S, Ida H, Eto Y. Reduced α -Gal A enzyme activity in Fabry fibroblast cells and Fabry mice tissues induced by serum from antibody positive patients with Fabry disease. *Mol Genet Metab.* 2008;94(3):313-318.
29. Benichou B, Goyal S, Sung C, et al. A retrospective analysis of the potential impact of IgG antibodies to agalsidase beta on efficacy during enzyme replacement therapy for Fabry disease. *Mol Genet Metab.* 2009;96(10):4-12. doi: 10.1016/j.ymgme.2008.10.004.

30. Linthorst GE, Hollak CE, Donker-Koopman WE, et al. Enzyme therapy for Fabry disease: neutralizing antibodies toward agalsidase alpha and beta. *Kidney Int.* 2004;66(4):1589-95.
31. Hollak CE, Linthorst GE. Immune response to enzyme replacement therapy in Fabry disease: impact on clinical outcome? *Mol Genet Metab.* 2009;96(1):1-3. doi: 10.1016/j.ymgme.2008.10.013.
32. Rombach SM, Aerts JM, Poorthuis BJ, et al. Long-term effect of antibodies against infused alpha-galactosidase A in Fabry disease on plasma and urinary (lyso)Gb3 reduction and treatment outcome. *PLoS One.* 2012;7(10):e47805. doi: 10.1371/journal.pone.0047805.
33. FDA, Guidance for Industry, Immunogenicity Assessment for Therapeutic Protein Products, 2014, August. Accessed July 30, 2015: <http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm338856.pdf>).
34. Barbier AJ, Bielefeld B, Whiteman DA, et al. The relationship between anti-idursulfase antibody status and safety and efficacy outcomes in attenuated Mucopolysaccharidosis II patients aged 5 years and older treated with intravenous idursulfase. *Mol Genet Metab.* 2013;110(3):303-10. doi: 10.1016/j.ymgme.2013.08.002.
35. Kim J, Park MR, Kim DS, et al. IgE-mediated anaphylaxis and allergic reactions to idursulfase in patients with Hunter syndrome. *Allergy.* 2013;68(6):796-802. doi: 10.1111/all.12155
36. Schiffmann R, Kopp JB, Austin HA 3rd, et al. Enzyme replacement therapy in Fabry disease: a randomized controlled trial. *JAMA.* 2001;285(21):2743-9. doi:10.1001/jama.285.21.2743.

37. Schiffmann R, Ries M, Timmons M, et al. Long-term therapy with agalsidase alfa for Fabry disease: safety and effects on renal function in a home infusion setting. *Nephrol Dial Transplant*. 2006;21(2):345-54. doi: 10.1093/ndt/gfi152
38. Ries M, Clarke JR, Whybra C, et al. Enzyme-replacement therapy with agalsidase alfa in children with Fabry disease. *Pediatrics*. 2006;118(3):924-32. doi: 10.1542/peds.2005-2895
39. FDA, Guidance for industry, Assay development for immunogenicity testing of therapeutic proteins, draft Guidance, 2009, December. Accessed July 30, 2015: <http://www.fda.gov/downloads/Drugs/Guidances/UCM192750.pdf>
40. Biomarkers definitions working group. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clin Pharmacol Ther*. 2001;69:89-95. Doi: 10.1067/mcp.2001.113989.
41. FDA, Guidance for industry, E16 biomarkers related to drug or biotechnology product development: Context, structure, and format of qualification submissions. 2011. Accessed October 30, 2015: <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM267449.pdf>
42. FDA, Guidance for industry and FDA staff. Qualification process for drug development tools. Accessed October 30, 2015: www.fda.gov/downloads/drugs/guidancecomplianceinformation/guidances/ucm230597.pdf

43. Kishnani PS, Nicolino M, Voit T, et al. Chinese hamster ovary cell-derived recombinant human acid alpha-glucosidase in infantile-onset Pompe disease. *J Pediatr*, 2006. 149(1): p. 89-97. doi:10.1016/j.jpeds.2006.02.035
44. Kishnani PS, Corzo D, Leslie ND, et al., Early treatment with alglucosidase alpha prolongs long-term survival of infants with Pompe disease. *Pediatr Res*, 2009. 66(3): p. 329-35. doi: 10.1203/PDR.0b013e3181b24e94.
45. Prater SN, Banugaria SG, DeArmev SM, et al. The emerging phenotype of long-term survivors with infantile Pompe disease. *Genet Med*. 2012;14(9)800-10. doi: 10.1038/gim.2012.44.
46. Prater SN, Patel TT, Buckley AF, et al. Skeletal muscle pathology of infantile Pompe disease during long-term enzyme replacement therapy. *Orphanet J Rare Dis*. 2013;8:90. doi: 10.1186/1750-1172-8-90.
47. Berrier KL, Kazi ZB, Prater SN, et al. CRIM-negative infantile Pompe disease: characterization of immune responses in patients treated with ERT monotherapy. *Genet Med*. 2015. Mar 5. doi: 10.1038/gim.2015.6.[Epub ahead of print].
48. Banugaria SG, Patel TT, Kishnani PS. Immune modulation in Pompe disease treated with enzyme replacement therapy. *Expert Rev Clin Immunol*. 2012;8(6):497-9. doi:10.1586/eci.12.40
49. Banugaria SG, Prater SN, Patel TT, et al. Algorithm for the early diagnosis and treatment of patients with cross reactive immunologic material-negative classic infantile pompe disease: a step towards improving the efficacy of ERT. *PLoS One*. 2013;8(6):e67052. doi: 10.1371/journal.pone.0067052.

50. Prater SN, Banugaria SG, Morgan C, et al. Letter to the editors: Concerning “CRIM-negative Pompe disease patients with satisfactory clinical outcomes on enzyme replacement therapy” by Al Khallaf et al. *J Inherit Metab Dis*. 2014;37(1):141-3. doi: 10.1007/s10545-013-9637-8.
51. Stenger EO, Kazi ZB, Lisi E, Gambello MJ, Kishnani PS. Immune tolerance strategies in siblings with infantile Pompe disease - Advantages for a preemptive approach to high-sustained antibody titers. *Molecular Genetics and Metabolism Reports*. 2015. doi: 10.1016/j.mgmr.2015.05.004.
52. Bali DS, Goldstein JL, Banugaria S, et al. Predicting cross-reactive immunological material (CRIM) status in Pompe disease using GAA mutations: lessons learned from 10 years of clinical laboratory testing experience. *Am J Med Genet C Semin Med Genet*. 2012;160C(1):40-9. doi: 10.1002/ajmg.c.31319.
53. Schwab I, Nimmerjahn F. Intravenous immunoglobulin therapy: how does IgG modulate the immune system? *Nat Rev Immunol*. 2013;13(3):176-89. doi: 10.1038/nri3401
54. Kim M, Martin ST, Townsend KR, Gabardi S. Antibody-mediated rejection in kidney transplantation: a review of pathophysiology, diagnosis, and treatment options. *Pharmacotherapy*. 2014;34(7):733-44. doi: 10.1002/phar.1426.
55. Banugaria SG, Prater SN, McGann JK, Feldman JD, Tannenbaum JA, Bailey C, et al. Bortezomib in the rapid reduction of high sustained antibody titers in disorders treated with therapeutic protein: lessons learned from Pompe disease. *Genetics in medicine : official journal of the American College of Medical Genetics*. 2013;15(2):123-31. doi: 10.1038/gim.2012.110. PubMed PMID: 23060045; PubMed Central PMCID: PMC3744338.

56. Matzner U, Matthes F, Weigelt C, et al. Non-inhibitory antibodies impede lysosomal storage reduction during enzyme replacement therapy of a lysosomal storage disease. *J Mol Med (Berl)*. 2008;86(4):433-42. doi: 10.1007/s00109-008-0309-3.
57. Turner ST, Hopwood JJ, Bond CS, Brooks DA. Immune response to enzyme replacement therapy: 4-sulfatase epitope reactivity of plasma antibodies from MPS VI cats. *Mol Genet Metab*. 1999;67(3):194-205.
58. Benson G, Auerswald G, Elezovic I, et al. Immune tolerance induction in patients with severe hemophilia with inhibitors: expert panel views and recommendations for clinical practice. *Eur J Haematol*. 2012;88(5):371-9. doi: 10.1111/j.1600-0609.2012.01754.x.
59. Stiefel M, Pinkwart C, Haase R, et al. [Immune tolerance induction with high-dose FVIII and pulsed intravenous immunoglobulin]. *Hamostaseologie*. 2010;30 Suppl 1:S119-21. [Article in German].
60. FDA. Drugs@FDA. Accessed May 19, 2015 at:
http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.Search_Drug_Name
61. Wang J, Lozier J, Johnson G, Kirshner S, Verthelyi D, Pariser A, Shores E, Rosenberg A. Neutralizing antibodies to therapeutic enzymes: considerations for testing, prevention and treatment. *Nat Biotechnol*. 2008;26(8): 901-8. doi:10.1038/nbt.1484.

Tables and Figures

Table 1: FDA approved ERTs for lysosomal storage diseases

Disease	Defective/deficient enzyme	Primary substrate accumulation	Enzyme replacement therapy	Time of approval by FDA
Gaucher Disease	β -glucosidase (glucocerebrosidase)	glucosylceramide	alglucerase (Ceredase [®]) ^a	April 1991
			imiglucerase (Cerezyme [®])	May 1994
			velaglucerase alfa (VPRIV [®])	February 2010
			taliglucerase alfa (Elelyso [™])	May 2012
Fabry Disease	α -galactosidase A	globotriaosylceramide	agalsidase beta (Fabrazyme [®])	April 2003
Pompe Disease	Acid α -glucosidase	glycogen	alglucosidase alfa (Myozyme [®])	April 2006
			alglucosidase alfa (Lumizyme [®])	May 2010
MPS I (Hurler, Hurler-Scheie, or Scheie Syndrome)	α -L-iduronidase	dermatan sulfate and heparan sulfate	laronidase (Aldurazyme [®])	April 2003
MPS II (Hunter Syndrome)	Iduronate-2-sulfatase	dermatan sulfate and heparan sulfate	idursulfase (Elaprase [®])	July 2006
MPS IVa (Morquio A)	N-acetylgalactosamine-6 sulfatase (GALNS)	keratan sulfate and chondroitin-6-sulfate	elosulfase alfa (Vimizim [®])	February 2014
MPS VI (Maroteaux-Lamy Syndrome)	N-acetylgalactosamine 4-sulfatase (arylsulfatase B)	dermatan sulfate	galsulfase (Naglazyme [™])	May 2005

Source: Drugs@FDA, accessed at <http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm> July 30, 2015 [60]

^aCeredase was discontinued from the market

Table 2: Infusion reactions and antibody formation with enzyme replacement therapy in LSDs

Disease		Recombinant enzyme: generic and trade name	Administered dose and schedule	Proportion with infusion- associated reactions	Proportion with IgG antibody formation ^a
Gaucher disease	Type 1	β -glucocerebrosidase: imiglucerase (Cerezyme, Genzyme Corporation)	Dosages range from 2.5 U/kg three times per week to 60U/kg biweekly	13.8%	15%
		β -glucocerebrosidase: velaglucerase alfa (VPRIV, Shire HGT)	60U/kg biweekly	52%	1.9%
		β -glucocerebrosidase: taliglucerase alfa (Elelyso, Pfizer)	60U/kg biweekly	29%	53%
Fabry disease	Classic and late onset	α -galactosidase A: agalsidase beta (Fabrazyme, Genzyme Corporation)	1.0 mg/kg biweekly	50 – 65%	68%
		α -galactosidase A: agalsidase alpha (Replagal, Shire HGT)#	0.2 mg/kg biweekly	52%	64%
MPS type I	Hurler, Hurler- Scheie and Scheie syndromes	A-L-Iduronidase: laronidase (Aldurazyme, BioMarin Pharmaceutical/Genzyme Corporation)	0.58 mg/kg weekly	32%	97%
MPS type II	Hunter syndrome, both severe and attenuated	Iduronate-2-sulfatase: Idursulfase (Elaprase, Shire HGT)	0.5 mg/kg weekly	15%	47%
Pompe disease	Infantile onset	Acid α -glucosidase: alglucosidase alfa (Myozyme, Genzyme Corporation)	20 mg/kg biweekly	51%	95%
	Infantile- and late-	Acid α -glucosidase: alglucosidase alfa	20 mg/kg	\geq 5%	100%

	onset	(Lumizyme, Genzyme Corporation)	biweekly		
--	-------	---------------------------------	----------	--	--

Source: Drugs@FDA, accessed at <http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm> July 30, 2015 [60].

^aThe incidence of antibody positivity may be influenced by a number of factors related to assay methodology, sample handling, timing of sample collection relative to dosing, concomitant medications, and underlying disease, so direct comparisons between therapies is not recommended.

#agalsidase alpha (Replagal) is not an approved drug in the United States

Table 3. Examples of pharmaceutical agents used for immune tolerization

Examples of Pharmaceutical Agents Used for Immune-Tolerization

Agent	Proposed Mechanism(s) in Immune Tolerance	Potential Adverse Event	Considerations
Cyclophosphamide	Alkylating agent, inhibits cell function	T- Leukopenia, neutropenia, hemorrhagic cystitis, Stevens-Johnson syndrome, toxic epidermal necrolysis, transitional bladder cell carcinoma, hematologic malignancies	
Methotrexate	Inhibition of T-cell activation	Leukopenia, rarely acute pneumonitis, pulmonary fibrosis, and renal function impairment	
Rituximab	Monoclonal antibody to CD20 on B-lymphocytes	Immune suppression with lymphocytopenia	
Bortezomib	Proteasome inhibitor, depletes short and long-lived plasma cells	Anemia, leukopenia, thrombocytopenia, peripheral neuropathy, constipation	

Azathioprine	Suppresses cell-mediated Myelosuppression, malignancies (e.g., skin hypersensitivities, alters cancers, most commonly squamous cell antibody production, carcinoma, hepatosplenic T-cell lymphoma, suppresses T-cell activity hepatobiliary carcinomas, mesenchymal more than B-cell activity) tumors)
Cyclosporine	Inhibits T-lymphocyte activity by binding to cyclophilin Nephrotoxicity, electrolyte abnormalities
Mycophenolate	Inhibits proliferative responses of T and B lymphocytes, suppresses antibody formation by B-cells Leukopenia, anemia, thrombocytopenia
Corticosteroids	Inhibition of B-cells and T-cells mediated immune responses, inhibits synthesis of cytokines involved in hypersensitivity reactions and macrophage activations, causes up-regulation of anti-inflammatory genes Increased infection risk, osteopenia/osteoporosis, impaired wound healing, osteonecrosis

Table 4. Clinical and laboratory data of a cohort of CRIM-negative infantile-onset Pompe disease patients previously treated with immune tolerance induction

	1	2	3	4	5	6	7
Gender	Female	Male	Female	Female	Female	Female	Female
Ethnicity	Hispanic	African Canadian	Caucasian	African American	African American	Asian	African American
Allele 1	c.2608C> T p.Arg870 X	c.546+2T> C	c.236_246del p.Pro79ArgfsX 13	c.525delT p.Glu176ArgfsX 45	c.2560C> T p.Arg854 X	c.525_526del TG	c.2560C> T p.Arg854 X
Allele 2	c.2608C> T p.Arg870 X	c.546+2T> C	c.236_246del p.Pro79ArgfsX 13	c.2560C>T p.Arg854X	c.2560C> T p.Arg854 X	c.525_526del TG	c.2560C> T p.Arg854 X
Age at Diagnosis	2.5 mo	2.5 mo	2.0 mo	0.3 mo (10 days)	3.0 mo	5.5 mo	3.0 mo
Age at start of ERT and ITI	3.0 mo	4.1 mo	2.4 mo	0.4 (12 days)	3.5 mo	6.5 mo	4.0 mo
Time from diagnosis to start of treatment (ERT and ITI)	0.5 mo	1.6 mo	0.4 mo	0.1 mo (2 days)	0.5 mo	1 mo	1 mo
ERT (alglucosida se alfa; 20 mg/kg every other week)	Yes	Yes	Yes	20 mg/kg weekly	Yes	Yes	Yes
Deviation from actual ITI regimen	IVIG: 1 dose during	Monthly IVIG started at	None	Methotrexate: X14 weeks	None	None	IVIG started at week

shown in Figure 5	ITI +2 doses after ITI	week 4		(total 42 doses)			4, 8 monthly doses +2 extra doses at 8 mo
Repeat ITI	No	No	No	No	Yes (1 additional cycle at week 35)	Yes (1 additional cycle at week 43)	No
Length of alglucosidase alpha treatment at database lock (in weeks)	101	92	89	70	59	51	48
Current age (as of January 2013)	127 weeks (29.3 mo)	121 weeks (28.7 mo)	111 weeks (25.8 mo)	84 weeks (19.5 mo)	86 weeks (20 mo)	90 weeks (21.3 mo)	65 week (15 mo)*
Adapted from: Banugaria SG et al., PLoS One [49]							
Abbreviations: ERT = enzyme replacement therapy; ITI = immune tolerance induction; mo = months; IVIG = intravenous immunoglobulin							
*Patient 7 died at the age of 15 months (48 weeks into ERT);							

From: Banugaria SG, et al., PLoS One [49]

Table 5. Laboratory and safety parameters of a cohort of CRIM-negative infantile-onset Pompe disease patients previously treated with immune tolerance induction

		1	2	3	4	5	6	7
Antibody titers	Baseline	0	0	0	0	0	0	0
	Peak (Week)	0	0	0	0	6400 (wk 31)	6400 (wk 23)	1600 (wk 39)
	Last titer (week)	0 (wk 101)	0 (wk 92)	0 (wk 89)	0 (wk 70)	3200 (wk 59)	6400 (wk 51)	800 (wk 46)*
Infection/hospitalization at or around ITI administration	None	None			None	None	None	None
Infusion Reactions (IAR)	Associated	One episode	None	None	None	One episode	One episode	None
CD19%	Baseline	Normal	Normal	Normal	Not done	Normal	Normal	Normal
	CD19% recovery (week)	Yes (wk 20)	Yes (wk 25)	Yes (wk 20)	Not done	↑CD19% (wk 20-30)	↑CD19% (wk 20-30)	Yes (wk 31)
	Last count (week)	Normal (wk 46)	Normal (wk 25)	Normal (wk 75)	Not done	↓Normal (2nd round ITI)	↓Normal (2nd round ITI)	Normal (wk 37)*

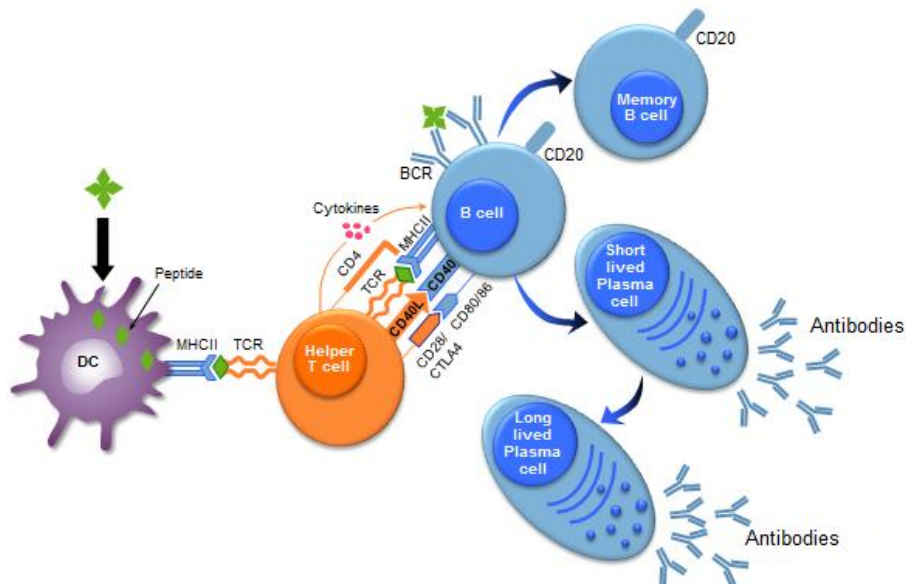
Vaccination status (Up-to-date except live vaccination)	Yes	Yes	Yes	Yes	Yes	Yes(till the start of ITI)	Yes
---	-----	-----	-----	-----	-----	----------------------------------	-----

*Patient 7 died at age 15 months (48 weeks into ERT) from disease progression unrelated to ITI administration

From: Banugaria SG et al. (2013) Algorithm for the Early Diagnosis and Treatment of Patients with Cross Reactive Immunologic Material-Negative Classic Infantile Pompe Disease: A Step towards Improving the Efficacy of ERT. PLoS ONE 8(6): e67052.
doi:10.1371/journal.pone.0067052 [49]

ACCEPTED MANUSCRIPT

Figure 1: Humoral immune response to a therapeutic protein

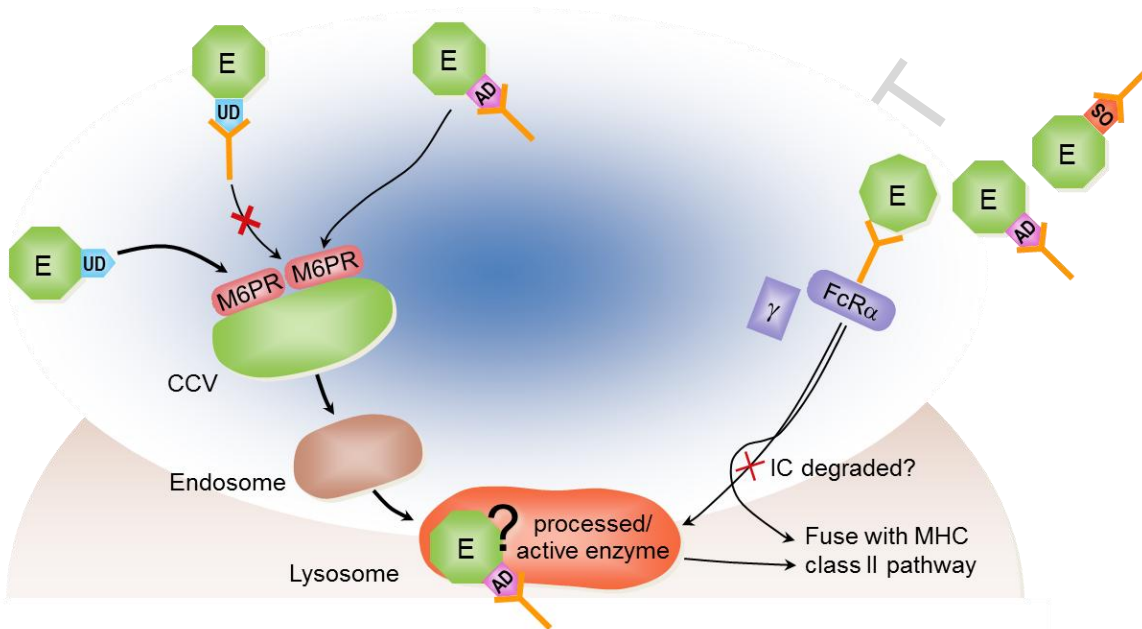


Legend: Generation of an antibody response to an enzyme therapeutic

✦ Therapeutic enzyme is taken up by antigen-specific B cells and dendritic cells, and peptides derived from the enzyme are generated and presented in association with MHC class II. MHC class II+ enzyme peptides engage helper T cells. Collaboration between helper T cells and enzyme-specific B cells (mediated by CD28, CD40 ligand and CD4 on the helper T cell with CD80/86, CD40 and MHC class II, respectively, on the B cell) induces maturation and differentiation of B cells to memory B cells, and antibody secreting short and, potentially, long-lived plasma cells

Abbreviations: MHC, Major Histocompatibility Complex; TCR, T cell receptor; BCR, B cell receptor; DC, dendritic cell.

Figure 2. Impact of neutralizing antibody on enzyme entry and activity in ERT



Modified from: Wang J, et al., Nat Biotechnol. 2008;26(8):901-908. [61]

Legend: Antibody may block ERT target cell entry and catalytic activity or facilitate enzyme uptake in nontarget cells

Impact of neutralizing antibody on enzyme entry and activity in ERT: There are several possible outcomes of the binding of lysosomal enzyme-specific antibodies (yellow 'Y'): blockade of enzyme uptake through M6PR by binding to the receptor binding (uptake domain(s) (UD); blockade of enzyme uptake by M6PR and suppression of enzymatic activity by binding to epitopes near the receptor binding domain and the enzymatic activity domain (AD; theoretical); blockade of both uptake and activity domains by separate antibodies specific for each site; degradation of the enzyme by catalytic antibody; reduction of enzymatic activity by targeting the enzymatic domain (AD); prevention of enzyme maturation by targeting the enzyme protease processing sites (not shown); and targeting of other sites (OS) of the enzyme, resulting in conformational or trafficking changes. Binding of antibodies to enzyme

may redirect the enzyme to FcR-expressing cells, such as macrophages and B cells. Enzyme– antibody complexes internalized through FcRs may prevent proper translocation of functional enzymes to the lysosome.

Abbreviations: AD, enzymatic activity domain; CCV, clathrin coated vesicles; E, enzyme; M6PR, mannose-6-phosphate receptor; MHC, Major Histocompatibility Complex; OS, other sites; UD, uptake domain; Y, specific antibodies

Figure 3. Case example, Lurie Children's Hospital (Burton BK), antibody titer decline after immune tolerance induction in a patient with Hunter syndrome

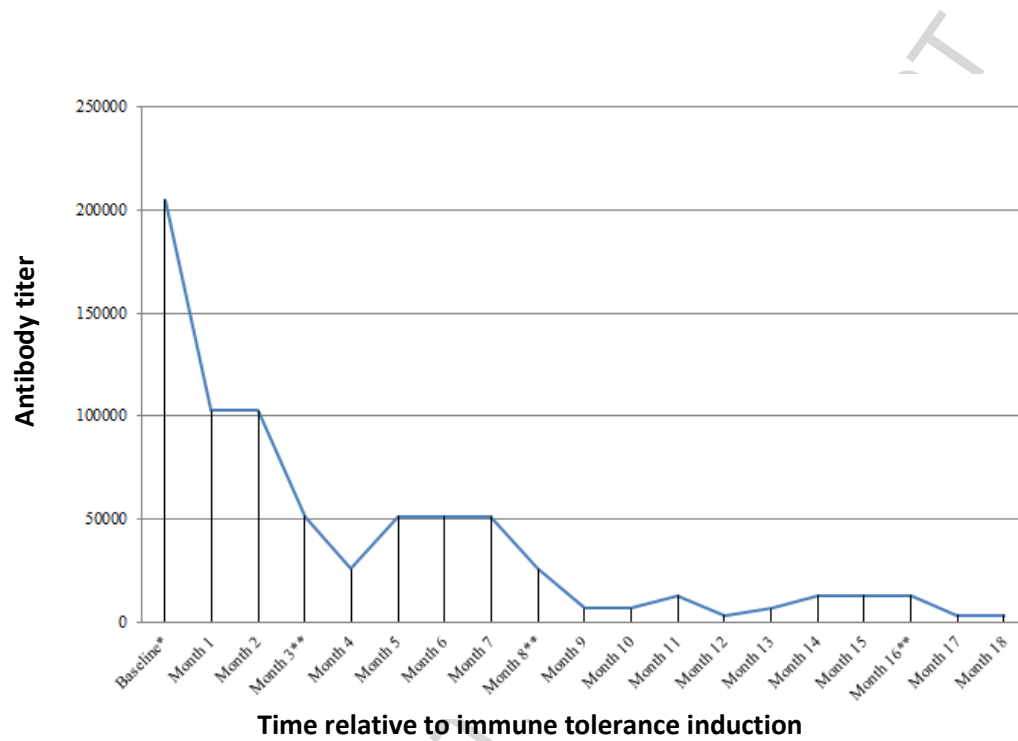
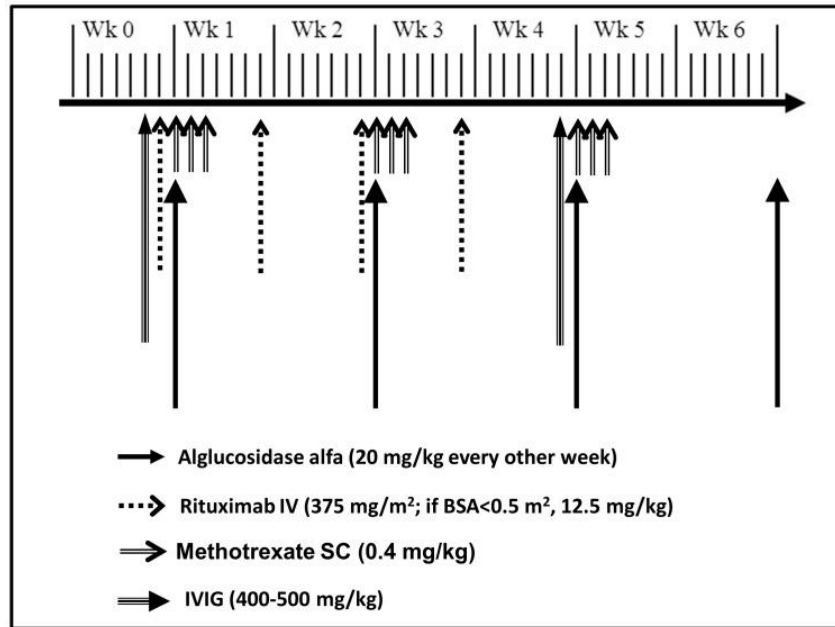
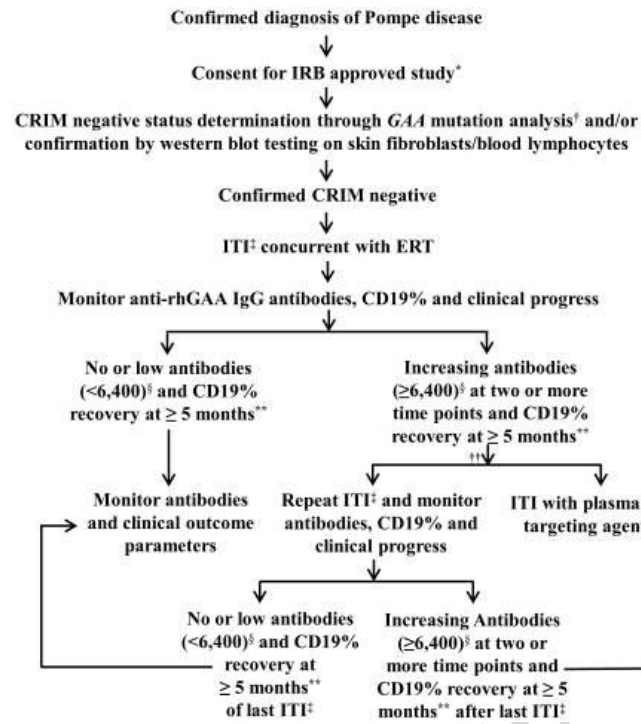


Figure 4. Immune tolerance induction regimen used in Duke experience in collaboration with treating physicians of CRIM-negative infantile-onset Pompe disease patients



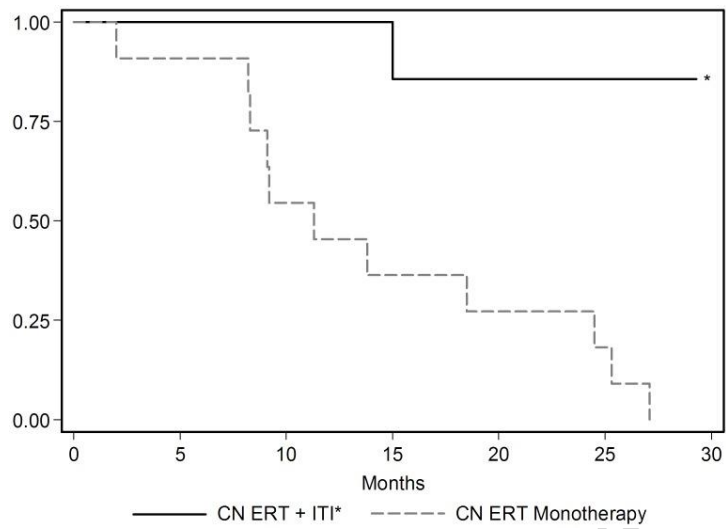
From: Banugaria SG, et al., PLoS One [49]

Figure 5. An algorithm for the management of CRIM-negative infantile Pompe disease patients



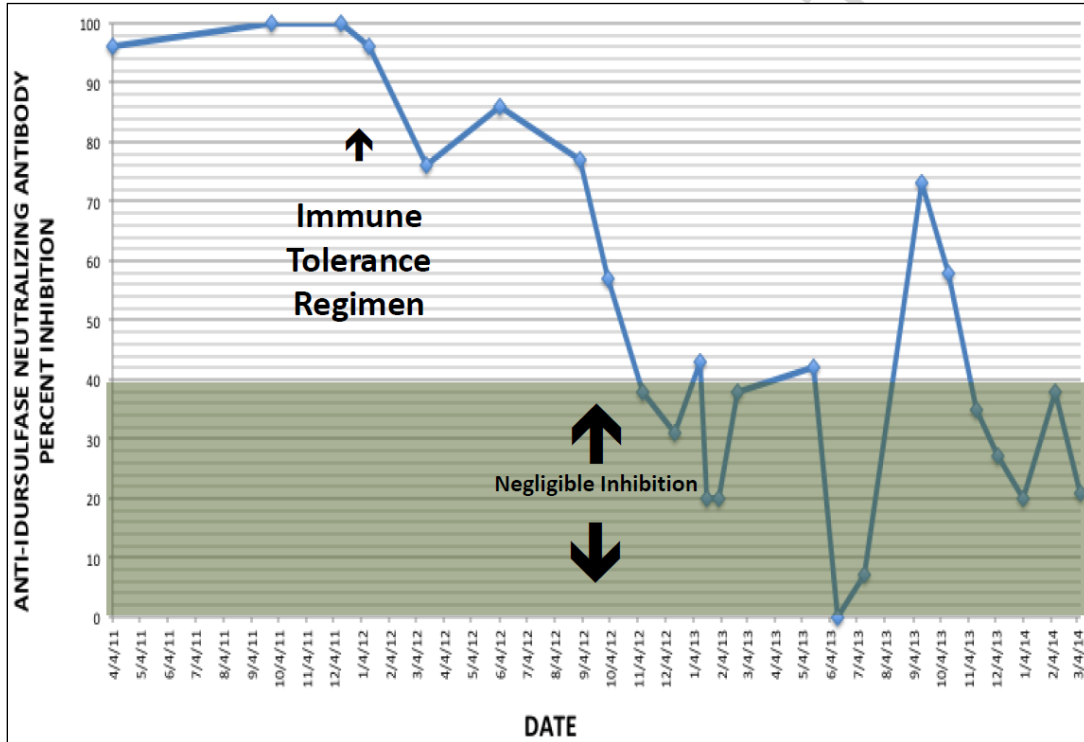
From: Banugaria SG, et al., PLoS One [49]

Figure 6. Kaplan-Meier survival curve showing comparison of ventilator-free survival CRIM-negative ERT monotherapy (n=11) versus ERT + ITI (n=7) treated patients [46]



From: Banugaria SG, et al., PLoS One [49]

Figure 7: Neutralizing antibody response to non-cytotoxic, non-immunosuppressing, immune-tolerizing regimen (IVIG and more frequent ERT dosing) in another patient with Hunter syndrome



Highlights

- Development of neutralizing anti-drug antibodies (ADAs) has been noted in patients receiving enzyme replacement therapies (ERTs) for lysosomal storage diseases
- Patients with more severe gene mutations (null mutations, large deletions) appear to be at greater risk of developing ADAs, which may result in poor clinical outcomes
- Entrenched ADA responses can be difficult to treat. Clinical experience in small numbers of high-risk patients have shown promising results with prophylactic immune tolerizing therapies to prevent ADA development using combination therapy with anti-B cell and anti-plasma cell drugs
- There is a need for more research into more sensitive outcome measures (e.g., biomarkers and clinical outcomes) to better monitor patients receiving ERTs over the long-term treatment in order to optimize therapeutic interventions