World*Symposium*™ 2023 Conference Review™
Lysosomal storage disorders (LSDs)

Making Education Easy | 22-26 February 2023, Orlando, Florida

In this review:

Abbreviations used in this review:

ALT = alanine aminotransferase;
ASMD = acid sphingomyelinase deficiency; **BMI** = body mass index;
CI = confidence interval; **CNS** = central nervous system;
HDL = high-density lipoprotein; **HR** = hazard ratio;
mRNA = messenger RNA; **MPS** = mucopolysaccharidosis;
PGRN = progranulin; **siRNA** = silencing RNA.

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Welcome to our review of the 19th Annual World*Symposium*™

held this year in Orlando, Florida, USA in February. The symposium aids researchers, clinicians, nurses and genetic counsellors to optimally manage patients with lysosomal diseases and identify the latest findings in the natural history of these diseases. For this review I have selected 11 presentations from the meeting that I believe to be of interest and relevance to local practice.

I hope you find this conference review interesting and the content useful in your clinical practice.

Kind Regards,

Dr Heidi Peters

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The abstracts include products and treatment approaches that are investigational and are not approved in any country. Laboratory or animal data does not necessarily predict clinical effects.

Drugging transcription factors with small activating RNAs: A novel approach for enhancing bone marrow therapy for monogenic rare diseases

Authors: Habib N et al.

Summary: This presentation examined the mechanism of action of RNA activation duplexes that upregulate the master regulator of myeloid cells (CEBPA), and which have shown beneficial immunomodulatory effects in >140 cancer patients. Upregulation of CEBPA directly upregulates downstream targets in immune oncology, but also has effects on enzyme deficiency through the *IDUA* gene that encodes alpha-L iduronidase, enabling lysosomal degradation of the glycosaminoglycans, dermatan and heparan sulfate. *IDUA* mutations lead to autosomal recessive disease mucopolysaccharidosis type I (MPS1). Despite bone marrow transplantation (BMT), where wild-type enzymes from donor cells replenish enzyme levels, there is often still residual disease as the level of iduronidase does not rise sufficiently. In animal models, a single IV dose of MTL-CEBPA enhanced *IDUA* in bone marrow cells for 4 weeks up to 2-fold and secreted iduronidase by up to 3-fold over wild-type levels. MTL-CEBPA supercharged myeloid cells had a 35% increased efficiency in reversing the accumulation of GAG complexes versus cross correction with normal myeloid cells.

Comment: The use of RNA technology delivered in nanoparticles, as a therapeutic modality is increasing momentum. To date this has mainly focused on mRNA and siRNA approaches. In this work the potential of small activating RNA molecules to target the transcriptional upregulation of target genes is becoming realised. What is exciting is that the therapy described could potentially have a role in treating MPS1 post bone marrow transplantation where there remains significant residual disease without alternative therapeutic options. The mechanism being to increase the level of enzyme from the transplanted unaffected cells both in the marrow and in plasma. It also offers potential of requiring less frequent treatment of monthly versus the weekly requirement of current enzyme therapy. By repurposing this therapy from its current use in hepatocellular carcinoma, where a history of safety data already exists, it will enable a faster timeframe to clinical use in this patient population.

Development of consensus guidelines for the clinical care of individuals with Sanfilippo syndrome

Authors: O'Neill C et al.

Summary: This presentation discussed the development of clinical guidelines for the treatment of the rare, lysosomal disorder Sanfilippo syndrome, which is characterised by accumulation of heparan sulphate, multisystemic disease and progressive neurodegeneration. A steering committee of global experts convened by patient advocacy groups developed these consensus guidelines, with the caregiver perspective integrated throughout the process. A total of 178 consensus statements were endorsed by the steering committee and developed into a manuscript. These guidelines were developed to improve consistency of care and access to care guidance for patients affected by Sanfilippo syndrome.

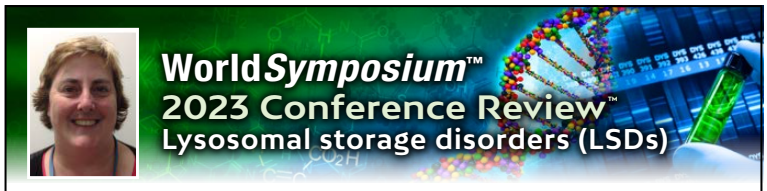
Comment: This presentation gave an outline of an international collaboration to develop the first set of published consensus clinical guidelines for Sanfilippo syndrome. A total of 178 statements were reached with greater than 75% consensus following broad consultation with a range of experts across a range of disciplines. As the authors highlight, such guidelines are important in enabling greater equity for patients and families in access to standardised care regardless of locality. They also provide a reference which can act as an enabler for families to access services/support and impact reimbursement schemes. It will be important to evaluate the effectiveness of the guideline in future, in addition to updating and modifying it as potential new therapies become available.

The spectrum of podocyte injury in later onset (LO) variants of Fabry disease (FD)

Authors: Najafian B et al.

Summary: This study examined podocyte injury in age-matched treatment-naive later onset Fabry disease (LOFD) and classic Fabry disease (CFD) patients aged 4-65 years based on kidney biopsies from 9 males and 6 females with LOFD, 9 males and 7 females with CFD, and 8 male and 5 female normal controls assessed using electron microscopic stereology. Globotriaosylceramide (GL3) inclusion volume per podocyte and podocyte volume increased and podocyte number density decreased with increasing age in both LOFD and CFD patients. Podocyte number density was reduced in male and female CFD and LOFD patients versus normal controls. Podocyte number density was approximately 4-fold lower ($p = 0.04$) in CFD patients aged ≤12 years versus normal controls and was correlated inversely with podocyte volume and inclusion volume per podocyte in both LOFD and CFD patients. Foot process width was greater in all Fabry disease patients versus normal controls but did not differ between Fabry disease groups. Glomerular filtration rate (GFR) was inversely correlated with foot process width (r -0.64; $p = 0.014$), podocyte volume (r -0.69; $p = 0.005$), and inclusion volume per podocyte (r -0.61; $p = 0.016$) in LOFD, but not CFD patients. Multivariate analysis in LOFD patients suggested that 54% of GFR variability was due to podocyte volume, inclusion volume per podocyte and foot process width ($p = 0.009$); foot process width was the only independent predictor of GFR.

Comment: See next summary



Independent commentary by Dr Heidi Peters

Dr Heidi Peters is a biochemical geneticist who works as a specialist in the metabolic unit at Genetic Health Services Victoria. She also heads research aimed at developing novel therapies for inborn errors of metabolism.

Sanofi have offered sponsorship for my virtual attendance.

Development of an online cloud-based tool for automatic measurement of foot process width (FPW) using deep learning (DL): Applications in assessment of podocyte injury in Fabry disease (FD)

Authors: Smerkous D et al.

Summary: This presentation outlined development of an online, cloud-based tool for the automatic identification and quantification of foot processes using deep learning based on a total of 7342 slit diaphragms and 8,152,303 nm of podocyte glomerular basement membrane interface (PGBMI) on electron microscopic (EM) images from biopsies of Fabry nephropathy patients and healthy controls. The model had a 61-67% dice score for slits and 76-86% for PGBMI; model precision performance on segmentation of 6960 filtration slits was 91% with a recall/sensitivity of 78%. In biopsies from 40 Fabry disease patients and 11 normal controls there was no difference between the deep learning model and human measured stereology, while comparison of 32 biopsies from CFD patients and 6 biopsies from LOFD patients also found no significant difference between foot process width with the 2 analyses. Deep learning identified progression of foot process width with increasing age and inverse relationship between foot process width and podocyte number density in CFD patients, but these relationships were not observed by human stereology.

Comment: These studies provided important information on the impact on podocytes of LOFD and compared the findings to CFD and controls. One of the most challenging findings from this study was that patients in the CFD <12 years of age, which included children as young as 4 years, already had a reduction in density of podocytes. This raises concern that if podocyte densities are already reduced at such a young age, what potential limitation current therapies have on actually being able to correct renal dysfunction. The study authors query whether this finding is due to very early podocyte loss or whether in fact it is a result of podocyte eugenesis during fetal development from an *in utero* impact from maternal condition. Due to a lack of good animal models, understanding the basis of this finding is hard to determine, however, the group have shown *in vitro* that *galactosidase alpha* gene (*GLA*) mutations reduce the capacity for podocytes to proliferate. The second presentation described the successful use of deep learning techniques to develop a rapid and reliable method to assess podocyte foot width, an early marker of podocyte injury in electron microscopy images. This reduced the time of assessing foot process width in an EM of kidney biopsy from 8 hours by manual methods to around 10 minutes using this tool. They have also developed a cloud-based site that allows access to the tool for public use and ease of future assessments.

Plasma lyso-sphingomyelin as a biomarker for acid sphingomyelinase deficiency: Correlations with baseline disease and response to olipudase alfa treatment in clinical trials

Authors: Wasserstein MP et al.

Summary: This pooled analysis of 3 clinical trials of olipudase alfa assessed the effect of high elevations of lyso-sphingomyelin in 20 untreated children and 41 adults with acid sphingomyelinase deficiency (ASMD), and correlated plasma lyso-sphingomyelin levels with indices of disease severity. Pre-treatment lyso-sphingomyelin ranged from 12-144-fold the upper limit of normal (ULN; 9.9 µg/L). Among adults, baseline correlations were generally moderate to strong between lyso-sphingomyelin and spleen volume in multiples of normal (MN; $r = 0.808$; $p \leq 0.0001$) and liver volume ($r = 0.670$; $p \leq 0.0001$), HDL cholesterol ($r = -0.554$; $p = 0.0002$), lung diffusing capacity for carbon monoxide (DL_{CO}) ($r = -0.436$; $p = 0.0049$), and ALT ($r = 0.630$; $p < 0.0001$). Among children, correlations were moderate to strong for liver volume ($r = 0.734$; $p = 0.0002$), HDL cholesterol ($r = -0.536$; $p = 0.0147$), DL_{CO} ($r = -0.700$; $p = 0.0358$), and ALT ($r = 0.518$; $p = 0.0192$). After olipudase alfa treatment, lyso-sphingomyelin levels decreased rapidly (>60%) within 3 months, plateauing after 9-12 months at 70-88% decrease, but, in most patients, did not fully normalise. Most clinical improvements were within 6 months of treatment but continued to improve over 2-7.5 years on treatment.

Comment: Olipudase alfa is the first enzyme therapy approved* for treatment of Niemann pick A/B. It is ideal to have a biomarker that correlates with clinical improvement in order to monitor effectiveness of therapy. This study assessed all the olipudase trials undertaken in paediatric and adult patients with Niemann Pick A/B and compared changes in clinical disease with levels of lyso-sphingomyelin. For adult patients there was a correlation between changes in liver, spleen volume and DL_{CO} function and with lyso-sphingomyelin. In children these correlations were also observed for all parameters other than spleen volume. There was no clear explanation for this observation, however, lyso-sphingomyelin seems to represent a reasonable monitoring biomarker.

**** approved in the US, Europe and Japan***

Long-term catch-up growth in children with acid sphingomyelinase deficiency treated with olipudase alfa enzyme replacement therapy in the ASCEND-Peds trial

Authors: Giugliani R et al.

Summary: This was a long-term follow-up to the multinational open-label ASCEND-Peds study in 20 children with ASMD after treatment for 2.5-5.7 years (mean 4.0 years). Height Z-score improved in all patients (least-square [LS] mean 2-year increase 1.17; $p < 0.0001$); with 10 children having short stature at baseline versus 1 at data cut-off. Across all patients, difference between actual age and bone age improved from a mean of -24.4 months at baseline to -17.0 months at 2 years (LS mean difference 8.6 months; $p = 0.0065$). After 2 years, spleen and liver volume decreased in all patients, while lung function, imaging, lipid profiles, liver function tests, and plasma biomarkers all improved. Most adverse events were mild or moderate; 4 patients experienced 7 treatment-related serious adverse events, none of which led to treatment discontinuation.

Comment: Niemann Pick A/B is a multi-system disease with the major disease burden resulting from hepatosplenomegaly, lung disease and poor growth. In this presentation the authors present the dramatic and sustained improvement in growth in children within the ASCEND-Peds trial. On commencing enzyme replacement treatment with olipudase alfa, growth parameters rapidly changed from below the 3rd percentile to within the average range with continued catch-up growth also observed in late adolescence into early twenties. This was accompanied by a resolution of delayed bone age. There was no data presented on weight or BMI and it would be interesting to observe if these also showed the same trend. It is assumed the growth is a result of overall disease improvement; however, measurement of levels of growth hormone and any changes in these pre- and post-treatment would be interesting to measure.

Defect-free graphene enhances enzyme delivery to fibroblasts derived from the patients with lysosomal disorders

Authors: Vranic S et al.

Summary: This laboratory study examined the effect of graphene-based materials (GBMs), including defect-free graphene flakes and graphene oxide (GO) with different lateral dimensions, as a platform for loading of therapeutic cargos in healthy and primary fibroblasts from the patients with MPS VI and Pompe disease. The study observed excellent biocompatibility of all GBMs up 100 µg/mL. In addition, a noticeable difference in the uptake profile of the materials was observed. Neither type of GO was taken up by any cell lines. Both negatively and positively charged defect-free graphene was efficiently taken up. Cationic defect-free graphene can be used as arylsulfatase B (ARSB) carriers with the Gr:ARSB complex retaining higher enzyme activity and a biological effect almost 2-fold as effective as ARSB for clearance of the substrate in MPS VI fibroblasts.

Comment: A major hurdle in optimising therapeutics is obtaining efficient and accurate delivery to the site where it is to be active. This remains a limitation of current enzyme therapies for lysosomal disorders to varying degrees with a clinical need to improve their efficacy. In this presentation, the authors describe the use of a number of 2D graphene structures onto which therapeutics are loaded and which then act as delivery vehicles. The recognition that such structures may be beneficial for treatment of a range of lysosomal disorders occurred coincidentally when the method failed to be effective for siRNA delivery as the graphene structures were trafficked to the lysosome. This led to a redirection of therapeutic focus which eventuated as a result of interactions between basic scientists and clinicians and highlights the importance of the close collaboration for cross fertilisation of ideas and knowledge of therapeutic need. This technology is now being investigated as a potential delivery method for enzyme therapies with initial data indicating lack of cell toxicity in fibroblast lines and improved delivery for certain disease specific enzymes. Significant questions remain as to which enzymes will be responsive and *in vivo* effects; however, it provides an exciting new potential approach with clinical applicability.

Changes in hematologic and visceral manifestations over time following imiglucerase initiation in Gaucher disease type 1 and type 3 pediatric patients in the ICGG Gaucher Registry

Authors: Mistry PK et al.

Summary: This analysis of data from the real-world, longitudinal, international ICGG Gaucher Registry database assessed the response of spleen volume, haemoglobin, and platelets in non-splenectomised paediatric 778 Gaucher disease type 1 (GD1) and 185 type 3 (GD3) patients receiving imiglucerase. Baseline median spleen volumes were 17.0 MN in GD1 and 35.3 MN in GD3 patients with haemoglobin levels 10.9 g/dL and 9.6 g/dL and platelets of 106.0 $x10^9$/L and 97.5 $x10^9$/L. Median age was 7.7 years at imiglucerase initiation in GD1 and 1.9 years in GD3 patients. Imiglucerase treatment reduced splenomegaly in GD1 patients at 1 (41.0%), 5 (66.6%) and 10 years (74.4%) and by 37.7%, 66.1% and 79.6% in GD3 patients. In GD1 patients, haemoglobin increased by 1.5 g/dL at 1 year, 1.9 g/dL at 5 years and 2.6 g/dL at 10 years, while in GD3 patients, haemoglobin increased by 2.0, 2.3 and 3.2 g/dL. In GD1 patients, platelet count increased by 45.8% at 1 year, 56.4% at 5 years and 65.8% at 10 years, while in GD3 patients, platelets increased by 72.6%, 91.5% and 116.8%.

Comment: Gaucher disease is a disorder with extreme phenotypic diversity, diversity in patterns of disease and in natural history. The extent of the clinical spectrum can be captured in well run international registries. Given the diversity it is also essential to understand the real-world experience of the impact of treatment in this diverse population as opposed to the more homogenous populations enrolled into pivotal trials. This study utilises the global Gaucher registry, which has been collecting data for 30 years, to assess and compare the response of children with GD1 and GD3 to imiglucerase treatment. The study was able to show a similar visceral and haematological pattern of response for both GD1 and GD3 to imiglucerase treatment, that continued over a prolonged timeframe. This study highlights the power of registry data, especially in rare disorders, to analyse a large cohort from a wide distribution of countries.

Building a better translational model of neuropathic Gaucher disease

Authors: Modi ME et al.

Summary: This study examined a mouse model of Gaucher disease with the D409V point mutation model for GD3 that used conduritol B epoxide (CBE) to further reduce lysosomal B-glucocerebrosidase (GCB) activity in the brain and exacerbate neurological phenotypes. Short-term CBE caused acute reduction of lysosomal GCB activity and glucosylsphingosine accumulation. The mice exhibited seizures and impaired function in several motor performance assays. The behavioural phenotypes accompanied increased brain expression of the microglial marker Iba-1 and lysosomal marker Lamp-1. Elevated glucosylsphingosine levels were maintained for ≥30 days after cessation of CBE administration and neurobehavioural deficits continued for ≥7 weeks. This model using short-term CBE in adult D409V animals allows for assessment of gene therapy approaches without compromising therapeutic transgene expression.

Comment: See next summary

A brain penetrant progranulin-derived biologic protects against neuronopathic Gaucher disease

Authors: Zhao X et al.

Summary: A second laboratory study examined the *in vivo* effect of lysosomal progranulin (PGRN) as a modifier of glucocerebrosidase in Gaucher disease by crossing mice without the glucocerebrosidase gene ($Grn^{-/-}$) with $Gba^{D409V/D409V}$ mice. These mice exhibited rapid accumulation of glucocerebrosidase substrates (glucosylceramide/glucosylsphingosine) in viscera and the CNS. Aged mice exhibited severe neurobehavioral deficits not observed in control mice and PGRN deficiency potentiated systematic inflammation. These mice also developed neurodegenerative phenotypes, with brain aggregation of α-Syn, TDP-43, and β-amyloid. A recombinant peptide derived from the PGRN c-terminal granulin E domain, crossed the blood-brain barrier, penetrated the brain, and increased glucocerebrosidase activity and reduced substrate accumulation. This peptide also reduced microgliosis and astrogliosis and prevented TDP-43 and α-Syn aggregation.

Comment: See next summary

Earlier-onset, more severe neurodegeneration in PGRN KO mice with a decreased dose of D409V *Gba1*

Authors: Lin Y et al.

Summary: In this study, the same research group examined the effect of a lower dose of the D409V *Gba1* gene crossed into *Grn* knockout mice ($Grn^{-/-}$;$Gba1^{D409V/Null}$), which exhibited an earlier onset and much more severe neurodegenerative disease. These mice had shorter life span (7.5 months) versus the 18 months of the previous mouse construct ($Grn^{-/-}$;$Gba1^{D409V/D409V}$). Compared to $Grn^{-/-}$;$Gba1^{D409V/D409V}$ mice and control mice ($Gba1^{D409V/Null}$ and $Grn^{-/-}$), $Grn^{-/-}$;$Gba1^{D409V/Null}$ mice developed worse short-term memory, abnormal gaits and hindlimb clasping, indicative of accelerated neurobehavioral deficits. By 7.5 months, these mice displayed robust increases in glucocerebrosidase substrates associated with more severe tissue inflammation ($CD68^{+}$) in viscera and CNS. In brain, spinal cord and retina, $Grn^{-/-}$;$Gba1^{D409V/Null}$ mice had more extensive astrogliosis and microgliosis than $Grn^{-/-}$;$Gba1^{D409V/D409V}$ and control mice. Notably, $Grn^{-/-}$;$Gba1^{D409V/Null}$ mice also developed overt Parkinson disease-like manifestations, including α-Syn and β-amyloid aggregates. Brain transcriptome profiling indicated a strong adverse relationship between CNS inflammation and immune response with *Gba1* dosage.

Comment: Animal models of neuronopathic Gaucher disease have been difficult to develop due to residual glucocerebrosidase activity in the brain. These studies report on several mouse models, developed using different methods, but which produce reasonable models of neuronopathic Gaucher. They show potential to be used for future therapeutic assessments including gene therapy. The first model utilises the D409V Gaucher model and administers the glucocerebrosidase inhibitor conduritol B epoxide to adult mice. The second and third group have crossed known Gaucher models with mice with genetic deficiency of progranulin, a glucocerebrosidase modifier. These mice are interesting in that a graded neurological disease severity could be obtained related to the severity of the Gaucher mutation in the cross. These authors also identified the critical binding fragment of progranulin (ND7), that when administered intravenously to the model, was able to cross the blood brain barrier and correct the neurological markers and reduce accumulated substrate. Excitingly the authors suggest ND7 has the potential to be a therapy to treat neuronopathic Gaucher disease and Parkinson's disease.

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