NATIONAL MPS SOCIETY MEMBERSHIP RESEARCH NEWSLETTER:

Contents

WORLD*Symposium*™ Recap

We hope you will enjoy this detailed recap of the 17th Annual WORLD*Symposium*™ held virtually February 8-12, 2021. This year's WORLD*Symposium*™ was extraordinary indeed. It featured impressive presentations of results from a number of clinical trials including for MPS I, MPS II, MPS IIIA, MPS IIIB. Many of these trials were initiated recently, while others were longstanding enough to document clear changes in neuropathic disease. Among the abstracts was also one of the first really encouraging approaches to treating ML II. These results represent truly momentous findings and progress for some of our most vexing clinical challenges.

While perhaps not as incredible as the results discussed above, there were also some pretty exciting studies with impact on the MPS and ML diseases, among which were ways to improve or limit the complications of immune responses to ERT or gene therapy. As always there were some interesting lessons from pre-clinical model work as well some intriguing findings in basic cell biology as well as studies of other lysosomal storage diseases that may point the way to improved approaches to address MPS and ML disorders.

A full treatment of the findings at WORLD*Symposium*™ can be found at
https://mpssociety.org/research-grants/worldsymposium-2021/

Clinical Trial and Clinical Updates

This was a truly exciting year to be at the WORLD*Symposium*™. There were a number of clinical and translational research updates all of which involved a very promising approach to treating both systemic and brain disease in the MPS disorders. Below are thematic recaps with links to overviews of listed study's details, followed by a sorted list of the same study's details.

Ex Vivo Lentiviral Gene Therapy, Hematopoietic Stem Cells and Transplantation.

- Uses a patient's own stem cells
- Cells are gene therapy treated outside the body with a lentiviral vector
    - Gene therapy treated cells then used to transplant the patient
- This could be a great improvement over routine transplantation
- May help address issues that limit transplant therapy in MPS II and the MPS III

- Summary of presentations
    - MPS I trial based in Milan (B. Genter, et al.)
    - Early but equally promising MPS IIIA trial based in Manchester (Kinsella et al.)
    - MPS II preclinical work in mice from the Univ. of Minn. and bluebird bio (Smith et al.)
    - Very powerful techniques to track gene therapy treated cells in patients post-transplant presented by AVROBIO (Baricordi et al.)

AAV Intrathecal Gene Therapy

- An approach to overcoming the blood brain barrier
- A balance of invasiveness (injection into cisterna magna) and efficiency (one-time treatment)

- One time gene therapy
- Direct injection via the cisterna magna, a space of CSF fluid at the top of the spine
- Delivers vector to the CSF bathing the brain and spinal cord

- Summary of presentations
  - Clinical trial updates on MPS II (Nevoret et al.) from REGENXBIO
    - Talks on methods of intracisternal injections in MPS I and MPS II (Pukenas et al.)
  - Preclinical MPS I mouse study looking at combine systemic and an intracisternal treatment
    - Presented by researchers at the Univ. of Minn. and REGENXBIO (Belur et al.).

IV Administered AAV Gene Therapy Designed to Treat the CNS

- The most long-standing studies of gene therapy in the MPS disorders
- A onetime IV administration of an AAV9 vector that also reaches cells of the brain
- The platform of Abeona's approach to treating both MPS IIIA and IIIB

- Summary of presentations:
  - In MPS IIIA encouraging results on patient measures of neurocognition (Flanigan et al.)
  - We are also starting to see results from the IIIB trial (de Castro et al.).

<u>Blood Brain Barrier (BBB) Transcytosis Engineered Enzyme</u>

- An approach that combines the safety and history of IV-ERT with BBB crossing technology
  - Designed to be able to treat neuropathic MPS disorders
  - The basis of the approach of Denali Therapeutics

- Summary of presentations:
  - The first clinical trial report of work from Denali on MPS II (Bakardjiev et al.)
  - Supporting studies from Denali of details on how their ERT crosses the BBB (Mahon et al.)
  - An MPS II mouse study from Denali documenting treatment benefits (Arguello et al.)

Intracerebroventricular (ICV) ERT

- ICV ERT involves ERT administered via an indwelling port into the brain ventricle
- An approach being evaluated in two clinical trials

- Summary of presentations:
  - A GC Pharma MPS II treating enzyme Hunterase® in ICV ERT (Okuyama et al.)
    - This therapy now approved in Japan!
  - The Allievex sponsored MPS IIIB clinical trial of tralesinidase alfa (AX 250)
    - An extremely important report on the longest running active trial in MPS IIIB
    - Muschol et al., presented positive results on patient clinical responses
    - Indicates how treatable MPS IIIB could be with early intervention

<u>Direct Intraparenchymal Brain Vector Administration</u>

- An approaches to overcoming the BBB

- Administers gene therapy vector surgically to brain tissue in a single surgery
- The champion of this approach to therapy is LYSOGENE

- Summary of presentations:
    - LYSOGENE clinical trial update for MPS IIIA with AAVrh10 vector (Hocquemiller et al.)
        - Update involved biochemical results for the CNS showing a positive response
        - Still too early in the trial to have a picture of the neurocognitive outcomes

Clinical Studies in Ultra Rare Diseases

- Ultra-rare diseases represent especially difficult diseases to study and to find treatments for.
- MPS VII is one such ultra-rare disease

- Summary of presentations:
    - Presentation of Dr. Marsden et al. on serious in utero and neonatal complication of MPS VII
        - Non-Immune Hydrops Fetalis (NIHF), a potential fatal early complication of MPS
        - This complication appears to be especially common in MPS VII
        - NIHF overview and its potential treatment with Mepsevii/vestronidase alfa ERT
        - Potential for ERT in utero

<u>Small Molecule Drug Studies and the Lessons of Other Lysosomal Storage Diseases</u>

- Small drug therapy could treat aspects of MPS and ML, with advantages including:
    - Small size of compounds, potentially approved drugs or drugs already proven safe, ability to reach the brain, and ability to improve a patient's enzyme activity or to stabilize ERT
- Small drugs may also serve as substrate deprivation therapy
    - Lowers the amount of substrate in the body, making the job of ERT easier

- Summary of presentations:
    - Late Onset Pompe Disease study (Schoser et al.) of an oral drug/chaperone for IV ERT
    - A substrate deprivation drugs for Gaucher showed benefit in the brain (Schiffmann et al.)

Overcoming The Immune Response for Improved ERT and Gene Therapy

- Immune response or antibodies may play a role in limiting the clinical benefit of ERT
- Pre-existing neutralizing antibodies to various AAV serotypes will limit the use of those serotypes
- As a result, immunomodulation is an attractive area of research

- Summary of presentations:
    - Desaie et al. presented positive results in Pompe patients on immune drug regimens for ERT
    - Choi et al. found that a drug combination for mice to overcome AAV neutralizing antibodies
    - Alexander et al. used an enzyme to treat mice and primates that chops up antibodies
        - Allows for a short-term window within which to treat with an AAV vector

Important Pre-Clinical and Translational Studies

New Approaches on the Horizon

- Innovations in proof of principle, pre-clinical, and translation research

- Summary of presentations:
    - Gotschall et al. reported on a therapeutic version of a missing enzyme in ML II and III
        - This is a huge development, as ML II and III are considered very difficult to treat
        - The new version of the enzyme appears safe in normal mice
    - Sigilon Therapeutics presented their encapsulated cell therapy (Tietz et al.) in MPS II mice
    - Ou et al., at the Univ of Minn., presented on CRISPR gene editing to treat MPS I mice
        - Used an approach similar to the Sangamo Therapeutics targeting the albumin gene
    - Researchers at the Children's Hospital of Orange County (Kan et al.) used human stem cells to successfully transplant human neural stem cells into MPS I mouse brains
    - Harm et al. at Iowa St. Univ. presented on the benefit of subcutaneous PPS treatment in canine MPS IIIB brains.

The Value of Basic Science in Better Understanding Disease

- Sometimes basic biology or physiology can be very important to guiding future research

- Summary of presentations:
    - Simonaro et al. presented on the endocannabinoid system (ECS) of the body
        - In mouse MPS IIIA, they found changes implicating the ECS with inflammation
        - Could prove useful in drug development targeting inflammation in MPS disease
    - Matthews et al. looked for differences across disease models for in neuroinflammation and neurodegeneration similar to diseases such as Parkinson's disease
        - MPS I mice had signs both general neurodegeneration and neuroinflammation
    - Seylani et al., at the NIH deleted a gene involved in lysosome formation in the liver of mice
        - "Reverse" engineering approaches in biology help scientists build robust and highly accurate models of how lysosomes function normally
        - Better understanding of this normal biology may help find MPS and ML treatments

Developing Better Clinical Tools

- For many of the MPS diseases, we are already at a place in our knowledge of how to treat them that the regulatory and approval process around clinical trials and drug approval are as limiting a factor to progress as the basic and translational science once were.
- Improvements in potential trial design can have a huge impact on fostering new treatment approval

- Summary or presentations:
    - Dr. Troy Lund and colleagues presented data CNS biomarkers that correlated with a positive response to therapy and improved neurocognitive outcomes.
        - Such validated biomarkers may speed testing of new and improved therapies

- A noninvasive brain imaging in a mouse model using resting state functional MRI or RS-fMRI, could help document response to therapy (Zhu et al. from the Univ. of Minn).
- Statistical approaches to study ultra-rare disease can improve the chance of documenting a positive outcome as we learned from Dr. Stepanians
- Sometimes it is not what you know but who you know that helps drive research in rare disease! Society member Jenny Klein and Society CEO Terri Klein co-authored an abstract on the Mucolipidosis Research Consortium Network (MRCN), a multi-institutional and multi-disciplinary network. They highlight the need for purposeful formation of research consortia in the field of rare disease research, to ensure excellent teams are created and function productively despite any challenges presented by distance or isolation.

Concluding remarks:

Jenny and Terri are engaged, as are all advocates and researchers, in a network to improve lives, which is what happens every day in the lives of the Society's members and supporters. Research is a vital part of that network, but equally, and perhaps more important are those networks that support us all in our everyday work to improve the lives and outcomes for our MPS and ML families and patients.

Sincerely,

Matthew Ellinwood

CSO of the National MPS Society

Studies by Syndrome

MPS I

MPS I (The San Raffaella Telethon Institute with collaborators and Orchard Therapeutics (OTL-203), NCT03488394)

Ex vivo hematopoietic stem cell gene therapy for mucopolysaccharidosis type I (Hurler syndrome). Bernhard Gentner at the San Raffaele Telethon Institute for Gene Therapy, Milan, and clinical trial partners.

Take homes:
- This is an update of an ongoing clinical trial for MPS I.
- This is similar to the approach being used in Manchester to treat MPS IIIA.
- Treatment involves combined gene therapy (OTL-203) and autologous hematopoietic cell transplant.
    - A patient's own cells (autologous) are harvested, gene therapy treated, and transplanted.
- To date eight patients have been treated successfully.
- Five patients that previously tested positive for antibodies, cleared these antibodies in three months.
- An average of a year and a half from treatment, all patients are alive and well.
- All have stable engraftment and roughly 1.4 gene therapy vector copies per white blood cell.
- Patients have at least over 3 fold normal enzyme blood levels.
- Patients have normalized urine GAG levels.
- Results support a CNS response due to microglia engraftment from gene therapy treated cells.

    - Iduronidase activity is detectable in the CSF.
    - There is a 20-fold decrease in heparan sulfate levels post treatment in the CSF.
- Patients with the longest follow-up have stable cognitive scores, with improved findings on brain and spine MRI, resumed growth velocity, and a lessening of their skeletal disease.
- This approach offers the potential to treat the CNS via high expressing gene therapy treated cells from the transplant migrating to the brain and becoming microglia cells.

MPS I (REGENXBIO, RGX-111, NCT03580083)

Intracisternal administration of AAV9 gene therapies to target the central nervous system. Bryan Pukenas at REGENXBIO clinical trial partners.

Take homes:

- This presentation covered both gene therapy for MPS I and MPS II and is listed here twice.
- The authors detailed the approach injection of REGEXBIO vector in MPS I.
- Various preclinical studies in model animals showed that intracisternal (into the CSF at the top of the spine), led to widespread dispersal of the gene therapy vector in the CNS.
- As part of patient treatment, they first undergo an MRI study.
    - This assesses the suitability of an injection into the CSF at the top of the spine.
    - If this approach is deemed viable, then it is used.
    - If it is not a via approach, a backup involves injection into the brain's lateral ventricle.
    - The lateral ventricle is a similar placement for shunts, hence a one-time injection there fits the model for other therapeutic approaches in the brain.
- The actual injection of vector into the intracisternal space at the top of the spine is done by a neurosurgeon or neuroradiologist with the guidance of a CT scan to ensure safety and accuracy of the injection.

MPS I Clinical Trial samples evaluated as biomarkers for neurocognition (NCT00638547).

Biochemical predictors of neurocognitive outcomes in Hurler syndrome. Troy Lund and colleagues at Univ of Minn and clinical partners.

Take homes:

- Biomarkers linked to and predictive of clinical response to therapy will help improve treatment.
- Spermine is a CNS biomarker that is associated with neuropathology and therapy response.
- Researchers evaluated spermine CSF of MPS I patients treated by transplant intrathecal ERT.
- Combined intrathecal ERT and stand of care transplantation led to lower CSF spermine levels.
- Correlations were drawn between spermine and neurocognition outcomes.
    - Positive correlation at two years after transplant.
- Spermine correlations with a behavior clinical benefit improves its utility as a biomarker.
    - This could lead to improved approaches to find and validate therapy improvements.

MPS II

MPS II (GC Pharma, Japanese intracerebroventricular Hunterase® clinical trail)

Prevention of cognitive decline in patients with neuronopathic mucopolysaccharidosis type II treated by intracerebroventricular enzyme replacement therapy: 100-week results of an open-label phase 1/2 study. Torayuki Okuyama at the National Center for Child Health and Development (Tokyo), and clinical trial partners.

Take homes:

- An ongoing trial for MPS II, using a delivery approach similar to the Allievex tralesinidase alfa trial.
- Hunterase® was well tolerated as an intracerebroventricular therapy every 4 weeks.
- CSF substrates were decreased 40-80% from baseline.
- Cognitive decline was prevented in the study, and better cognitive development was seen in patients enrolled before the age of three years.
- Updates post-WORLD*Symposium*™.
    - This product and approach have now been licensed to Clinigen K.K. as an approved therapy for MPS II in Japan.
    - A full publication associated with this presentation is now available.

MPS II (REGENXBIO, RGX-121, NCT03566043)

RGX-121 gene therapy for severe mucopolysaccharidosis type II (MPS II): Interim results of an ongoing first in human trial. Marie-Laure Nevoret at REGENXBIO, and clinical trial partners.

Take homes:

- This phase I/II clinical trial uses a viral vector (AAV9) to treat severe MPS II patients between four months and five years of age.
- Patients are treated once with a vector injection via a spinal tap in the upper neck.
- This is a dose escalation trial, with three increasing dose cohorts.
- Six patients treated to date as part of the first two dose cohorts.
- Treatments appear safe and well tolerated.
- Encouraging signs of therapeutic response in the brain.
    - The CSF biomarker shows reductions out to two years from treatment.
    - Patients experienced continued cognitive development and skill acquisition.
- Vector treatment also appears to affect systemic disease; plasma enzyme detected, and reduction in size in organ patients that were naïve to therapy pre-gene therapy treatment.
- Updates post-WORLD*Symposium*™.
    - REGENEXBIO has announced that they have treated the first patient at the highest dose in this dose escalation trial.

MPS II (REGENXBIO, RGX-121, NCT03566043)

Intracisternal administration of AAV9 gene therapies to target the central nervous system. Bryan Pukenas at REGENXBIO clinical trial partners.

Take homes:

- This presentation covered both gene therapy for MPS I and MPS II and is listed here twice.
- The authors detailed the approach injection of REGEXBIO vector in MPS I.
- Various preclinical studies in model animals showed that intracisternal (into the CSF at the top of the spine), led to widespread dispersal of the gene therapy vector in the CNS.
- As part of patient treatment, they first undergo an MRI study.
    - This assesses the suitability of an injection into the CSF at the top of the spine.
    - If this approach is deemed viable, then it is used.
    - If it is not a via approach, a backup involves injection into the brain's lateral ventricle.
    - The lateral ventricle is a similar placement for shunts, hence a one-time injection there fits the model for other therapeutic approaches in the brain.
- The actual injection of vector into the intracisternal space at the top of the spine is done by a neurosurgeon or neuroradiologist with the guidance of a CT scan to ensure safety and accuracy of the injection.

MPS II (Denali Therapeutics, DNL310, NCT04251026)

Intravenous ETV:IDS (DNL310) significantly reduces cerebrospinal fluid heparan sulfate in an open label Ph1/2 study in MPS II patients. Anna I. Bakardjiev at Denali Therapeutics, and clinical trial partners.

Take homes:

- This is an ERT based study designed to treat brain disease with an IV administration of ERT.
    - The drug DNL310 uses an Enzyme Transport Vehicle:IDS (ETV:IDS), used to carry the IDS enzyme across the blood brain barrier.
- The trial is a dose escalation study with primary safety and secondary efficacy endpoints.
- Five neuropathic MPS II patients have been enrolled.
- Initial safety data supported successful dose escalation (safe to increase dose).
- Biochemical endpoints were improved, as seen by biochemical signs of disease being reduced systemically (urine GAGs reduced) and in the CNS.
- CSF HS was normalized in 4/5 patients after 4 doses and was sustained out to 13 doses.
- These results are especially exciting because the ETV portion of the treatment can be coupled to other enzymes, opening the possibility of application to other MPSs.

MPS III

MPS IIIA (The University of Manchester, with collaborators and Orchard Therapeutics (OTL-201), NCT04201405)

Ex-vivo autologous stem cell gene therapy clinical trial for mucopolysaccharidosis type IIIA: Update on phase I/II clinical trial. Jane L. Kinsella (young investigator awardee) and colleagues at the Royal Manchester Children's Hospital and clinical trial

Take homes:

- This is an update of an ongoing clinical trial for MPS IIIA.
- This is similar to the approach being used in Milan to treat MPS I.
- Treatment involves combined gene therapy (OTL-201) and autologous hematopoietic cell transplant.

    - A patient's own cells (autologous) are harvested, gene therapy treated, and transplanted.
- To date three patients have been treated successfully.
- Early results (two months post-transplant), found WBC enzyme levels at over 150 fold normal.
- This approach offers the potential to treat the CNS via high expressing gene therapy treated cells from the transplant migrating to the brain and becoming microglia cells.

MPS IIIA (Abeona Therapeutics, ABO-102, NCT02716246 and NCT04360265 (long term follow up))

Updated results of Transpher A, a multicenter, single-dose, phase 1/2 clinical trial of ABO-102 gene therapy for Sanfilippo syndrome type A (MPS IIIA). Kevin M. Flanigan and clinical trial partners and colleagues at Abeona Therapeutics

Take homes:
- This is an update of the earliest gene therapy trial in any MPS III (start date March 2016).
- Treatment involves a one time IV administration of an AAV9 vector to treat MPS IIIA.
- The basis of CNS treatment involves AAV9's ability to access the brain and brain blood vessels.
- Termed Transfer A, this is a phase I/II, single IV treatment, escalating dose study.
    - Long-term patients roll over to an extension study after 24 months.
- Treatment was well tolerated by patients at all dose levels.
- Treatment at the highest dose leads to a rapid and significant drop in heparan sulfate in the CSF.
    - Significant improvements were also seen in liver size and plasma and urine GAG.
- Patients treated at ≤30 months of age at the highest dose had continuous gain of skill and cognitive function, assessed at up to 36 months post therapy.
- One patient treated at a year of age at the high dose has a developmental quotient of 100 (i.e. normal) at 2.5 years post-therapy.
- Overall, the results show impressive benefit in patients treated early at the highest dose.

MPS IIIA (LYSOGENE, LYS-SAF302, NCT03612869)

CNS-specific reductions of heparan sulfate and secondary storage biomarkers in Sanfilippo syndrome type A patients treated with the investigational gene therapy LYS-SAF302. Michaël Hocquemiller and clinical trial partners, and colleagues at LYSOGENE

Take homes:
- This trial (AAVance trial) is a phase II/III trial using a vector based on AAVrh.10 (LYS-SAF302).
- The trail is enrolled with 19 patients (most recent treated March 2020).
- Measurements of heparan sulfate and ganglioside levels in the CSF strongly support an active and therapeutic brain expression of therapeutic enzyme.
    - The biochemical responses seen in CNS as measured from the CSF are encouraging.
- Follow-up after greater time on trial will allow assessments of the primary outcome of Developmental Quotient (DQ) reflecting neurocognitive and neurodevelopmental assessments.

MPS IIIB (Abeona Therapeutics, ABO-101, NCT03315182)

Updated results of Transpher B, a multicenter, single-dose, phase 1/2 clinical trial of ABO-101 gene therapy for Sanfilippo syndrome type B (MPS IIIB). Maria J. de Castro and clinical trial partners, and colleagues at Abeona Therapeutics.

Take homes:

- This is an update of the MPS IIIB trial of Abeona's using an IV administered AAV9 viral vector.
- Substantially similar in design to the Abeona Transfer A study on AAV9 in MPS IIIA.
- Treatment involves a one time IV administration of an AAV9 vector to treat MPS IIIB.
- The basis of CNS treatment involves AAV9's ability to access the brain and brain blood vessels.
- Termed Transfer B, this is a phase I/II, single IV treatment, escalating dose study.
    - Long-term patients roll over to an extension study after 24 months (n=1 from cohort to date).
- Treatment infusion were well tolerated in all treated patients:
    - n=12 total: n=2 low dose, n=5 middle dose, n=4 high dose)
- One drug-related adverse event.
    - Prolonged hospitalization post-treatment of high dose patient to monitor vomiting and fever.
- Treatment let to a rapid (within one week) and sustained (out to 6 months in the high doses) normalized enzyme blood levels.
- Systemic improvements including decreased liver size and decreases in plasma and urine suberates.
- Treatment let to a rapid (within 30 days) and sustained (out to 2 years in the single patient to reach this time point) drop in CSF heparan sulfate).
- The limited duration since treatment, the low numbers, and slow disease progression limit any firm conclusions on the response of therapy seen in neurodevelopment and neurocognitive function.
- In summary, treatment appears well tolerated, and leads to biochemical therapeutic response measured in the systemic tissues and fluids, as well as in the CSF.
    - Further time is needed to assess any response in neurodevelopment and neurocognition.

MPS IIIB (Allievex, AX 250, NCT02754076 and extension study NCT03784287)

Tralesinidase alfa (AX 250) enzyme replacement therapy for Sanfilippo syndrome type B. Nicole Muschol and clinical trial partners, and colleagues at Allievex.

Take homes:

- This approach uses an intracerebroventricular (ICV) port to deliver recombinant enzyme for MPS IIIB directly to the ventricular system of the brain.
- This a phase I/II trial with 22 patients currently in a phase II study extension.
- Patients are treated weekly via the ICV port under the skin on the head.
    - The port is implanted via a one-time surgery.
- Patients have been followed out to 4 ½ years post therapy initiation.
    - Adverse events are consistent with those previously reported for ICV ports and ERT.
- Treatment normalizes MPS IIIB disease specific heparan sulfate fragments levels in 95% of patients.
    - Liver and spleen sizes were normal in most patients, indicating systemic effect of ICV doses.
- Multiple developmental, sensory, and behavioral parameters were improved or stabilized including:
    - Cerebral brain thickness increases (versus decline in untreated) if on therapy over 2 years.
    - Near universal improvements in hearing thresholds compared to before treatment.
    - Improved or stabilized cognition, adaptive behavior, sleep, and syndrome specific behavioral challenges.
- This trial is the first unequivocal results CNS enzyme delivery has a therapeutic effect in MPS IIIB.

MPS VII

MPS VII (Ultragenyx Pharmaceutical Inc., (Mepsevii/vestronidase alfa))

Significant unmet need in infants with mucopolysaccharidosis type VII and non-immune hydrops fetalis: A summary of cases. Deborah Marsden, and colleagues at Ultragenyx Pharmaceutical Inc.

Take homes:

- Non-immune hydrops fetalis (NIHF) is a serious in utero/neonatal complication seen MPSs, and especially in MPS VII.
- Researchers presented findings on up to 25 MPS VII patients with NIHF.
    - There was a 60% fatality rate which included in utero deaths.
    - 50% of cases succumbed to NIHF, before they could start ERT.
    - 40% of the cases who received ERT survived beyond one year.
    - 24% of patients with NIHF initiated ERT before age 1.
- In summary, NIHF is a potentially fatal condition associated with MPS VII.
- Better diagnosis and early initiation of treatment may better treat MPS VII NIHF patients.
- In utero therapy, not yet practiced for MPS VII NIHF is a technically viable option.

Pre-clinical and disease pathobiology updates

ML II

ML II gene therapy approach evaluated in normal mice (M6P Therapuetics)

Mucolipidosis type II AAV9 gene therapy pilot study: In vivo safety of over-expressing modified GlcNAc-1-phosphotransferase (S1S3) in wild-type mice
Robert R. Gotschall and colleagues at M6P Therapeutics.

Take homes:

- It was traditionally thought that gene therapy had limited potential to treat ML II/III because of the non-soluble nature of the missing enzyme.
- Using approaches to genetic engineering of the missing enzyme, scientists developed a vastly more efficient enzyme (designated as the S1S3 form).
    - With a non-soluble enzyme of improved efficiency, gene therapy may be viable.
- This study treated normal mice and evaluated them for toxicity and morbidity and mortality.
    - AAV treated mice did not show any toxicities of the gene therapy.
- The next step is to use this vector in the ML II mouse model.
- This is a huge step on the path to ML II therapy development.

MPS I

MPS I mouse brain imaging

Functional connectivity alterations in MPS I mouse brain at the laminar level revealed by resting-state fMRI. Wie Zhu (young investigator awardee) and colleagues at the Univ. of MN.

Take homes:

- MPS I mice were imaged non-invasively with MRI.
    - A special type of MPI called resting-state functional MRI (RS-fMRI).
    - Can evaluate brain structure non-invasively.
- MPS I mouse brains show regional changes affecting memory, sensory integration, and higher cognitive function.
- A critical finding that may help us understand MPS I and related MPS neuropathology.
- May lead to a better understanding of cognitive and behavioral characteristics of disease.
- May become a non-invasive technique to stage patients and gauge response to therapy.

MPS I mice transplanted with neural stem cells

iPSC-derived human neural stem cells engraft in the brains of immunocompromised MPS I mice. Shih-hsin Kan and colleagues at the Children's Hospital of Orange County.

Take homes:

- Researchers implanted MPS I mice with human neural stem cells.
    - Mice were genetically immunodeficient, so they didn't reject the human cells.
    - The stem cells were programmed from so called induced pluripotent stem cells (iPSCs).
- Researchers found substantial partial correction 8 months post transplantation.
- Exciting results but there remain many challenges before this could lead to human therapies so additional research in this area is indicated.

MPS I mice treated with a gene editing technology

Immunogenicity, genotoxicity, and efficacy of PS gene editing in treating MPS I mice. Li Ou and colleagues at the Univ. of MN.

Take homes:

- This is approach that includes AAV vectors, and the gene editing technology CRISPR.
- It is similar to the Sangamo Therapeutics zinc finger based approach.
- The work in the MPS I mouse model was both safe and effective.
- Basically, the AAV vectors deliver the gene editing tools of CRISPR to the liver.
    - These tools splice the iduronidase enzyme gene within the albumin locus.
- From the albumin locus in the genome iduronidase is produced at very high levels.
- This approach may be an improvement over zinc finger proteins-based gene editing.
- Results improved both brain and systemic signs, and initial work appears safe.
- Critical in ongoing studies is to examine the so called off target effects of gene editing.
- Off target mutations will be critical to evaluate for long term safety perspective.

MPS I mice treated with AAV9 gene therapy via IV, intracisternal, and combined delivery

Comparative systemic and neurologic effectiveness of intravenous and intrathecal AAV9 delivered individually or combined in a murine model of mucopolysaccharidosis type I. Lalitha Belur and colleagues at the Univ of MN and REGENXBIO.

Take homes:

- In brief, this is an animal model (mouse) extension and elaboration of the approaches used in the REGENXBIO AAV9 trials.
- Animals were treated by IV, intrathecal, or combined AAV9 gene therapy for MPS I.
- All three approaches resulted in therapeutic response in the mouse brain and periphery.
- Highest brain enzyme levels were seen with intrathecal therapy (10 times normal).
- however, all three treatments resulted in high levels of enzyme activity in major organs.
- In all treatment groups, neurocognitive deficits were absent.
- These results support the science behind the current human clinical trial.

MPS I mice and signs of neurodegeneration and neuroinflammation

Murine models of lysosomal diseases exhibit differences in brain protein aggregation and neuroinflammation. Jennifer Clarke Matthews and colleagues at Sanofi.

Take homes:

- Researchers studied neurodegenerative disease brain pathology similar to that of Parkinson's
- Many lysosomal storage diseases were studied.
- MPS I mouse brains were examined, and they had signs associated with proteinopathies and inflammation, both findings that are somewhat related to Parkinson's disease.
- Advanced CNS disease in MPS disorders may have many similarities of age-related neurodegenerative diseases in humans such as Parkinson's disease.

MPS II

MPS II ex vivo lentiviral gene therapy and HSCT in MPS II mice (Bluebird Bio)

Ex vivo lentiviral transduction of hematopoietic stem cells in mucopolysaccharidosis type II (MPS II) mice achieves high levels of systemic iduronate-2-sulfatase (IDS) enzyme activity and normalization of glycosaminoglycans (GAGs). Miles C. Smith (young investigator awardee) and colleagues at the Univ of Minn and bluebird bio, Inc.

Take homes:

- In brief, this is an animal study that uses a very similar approach to treat MPS II as is being trialed in human MPS I and MPS IIIA patients.
- The Milan study of MPS I and the Manchester study of MPS IIIA both used:
    - Transplantation with ex vivo gene lentiviral transduce hematopoietic stem cells.
- Transplanted mice had up to 100 X normal enzyme levels.
- Signs of disease storage were treated in the periphery and the brain.
- Treated mice had normal behavior on the tested platforms.
- These mouse results and those in other MPS human clinical trials support this approach to treatment of MPS II.

MPS II therapy with implantation of encapsulated cells (Sigilon Therapeutics, SIG-018)

SIG-018: Novel encapsulated non-viral cell-based therapy for MPS II. Drew Tietz and colleagues at Sigilon Therapeutics.

Take homes:

- This research tested an encapsulated cell system to treat MPS II mice.
- The cell encapsulation formula prevents immune system attack and fibrosis response that would wall off the cells form the body thus limiting therapy.
- Encapsulated human cells are engineered to produce high amounts of enzyme to treat MPS II.
- This could be a useful approach to treat MPS II.
- Tests in MPS II mice were presented and that treatment lowered GAG in tissues and body fluids.
- This drug candidate (SIG-018) could be a viable alternative to conventional ERT for MPS II.

Studies on Blood Brain Barrier crossing IV enzymes in MPS II mice

Molecular architecture determines brain delivery of transferrin receptor targeted iduronate 2 sulfatase in a mouse model of mucopolysaccharidosis type II. Cathal S. Mahon and colleagues at Denali Therapeutics, Inc.

Take homes:

- This research both studied and supported the approach of Denali Therapeutics and their MPS II therapy DNL310.
- Different transferrin receptor binding approaches to crossing the blood brain barrier were tested.
    - They used a mouse model of MPS II that expresses the human transferrin receptor.
- The transferrin mediated brain delivery used in DNL310 led to superior brain penetration.
    - Resulted in higher enzyme levels, more widely distributed and with GAG reductions.
- Other tested approaches resulted in less brain penetration and with enzyme being trapped in the brain blood vessels.

MPS II mice treated with a blood brain barrier crossing Enzyme Transport Vehicle:IDS (ETV:IDS)

Iduronate-2-sulfatase transport vehicle rescues neurobehavioral and skeletal phenotypes in a mouse model of mucopolysaccharidosis type II. Annie Arguello and research partners and colleagues Denali Therapeutics, Inc. and research partners.

Take homes:

- This research both studied and supported the approach of Denali Therapeutics behind their MPS II therapy DNL310.
- They used a mouse model of MPS II that expresses the human transferrin receptor.
- IV treatment with the brain penetrating formulation of enzyme led to decreased systemic and CNS disease.
- Biomarkers of brain disease (neurofilament light chain) were decreased in blood with IV ERT.
- Other treatment benefits included motor skills, sensorimotor gating, and learning and memory.
- These finding support the Denali platform and the human trials of DNL310.

MPS III

MPS IIIA mouse studies of the endocannabinoid system

Modulation of the endocannabinoid receptor CB2 as a novel treatment for the lysosomal diseases. **Calogera M. Simonaro and colleagues at the Icahn School of Medicine at Mount Sinai, New York.**

Take home

- The endocannabinoid system (ECS) is the body's physiological system that involves responses to cannabidiol oil and other cannabis products.
- It governs basic but incompletely characterized aspects of the nervous system and the immune and inflammatory response.
- The CB2 receptor mediates the immune and inflammatory response of the ECS.
- Researchers found an upregulation of the CBD system in MPS IIIA mouse liver, and found this upregulation decreased with anti-inflammatory medication.
- The changes in the CB2 system were also seen in another lysosomal storage disease model, supporting the idea this is a phenomenon that is seen in lysosomal storage diseases.
    - Findings MPS IIIA may hence be more generalizable to other MPS and ML disorders.

MPS IIIB Study on PPS treatment of the CNS in a dog model

Treatment with pentosan polysulfate improves neuropathological measures in the canine model of MPS IIIB. Tyler Harm (young investigator awardee) and colleagues at ISU.

Take home

- The treatment of MPS IIIB dogs reduces signs of brain inflammation and lysosomal storage.
- This despite the treatment being administered subcutaneously.
- May prove an important adjunct to enzyme-based therapy.
- An approved drug in Europe, this is a drug repurposing study in part.
- Part of a society funded research project, with researchers combining gene and PPS therapy.

Improving clinical efficacy with immune modulation approaches for ERT and gene therapy

Trial NCT01665326 in CRIM-negative Pompe disease patients involving ERT and immune tolerance

Transforming the clinical outcomes in CRIM-negative infantile Pompe disease identified via newborn screening: The benefits of early treatment with enzyme replacement therapy and immune tolerance induction. Ankit K. Desai at Duke University and clinical partners.

Take homes:

- Optimizing ERT in the newborn period could have big clinical impacts.
- Immune responses to ERT, especially in patients with no enzyme protein (so called CRIM-negative (CRIM(-)) could negatively impact early responses to therapy, and limit future benefits.
- This study evaluated approaches to prevent immune responses to ERT in CRIM(-) newborns.
- Researchers used three clinically approved drugs in the immune tolerance regimen.
- Initiation of treatment earlier in the course of disease improved outcomes.

- With the advent of newborn screening for MPS I and II, these sorts of studies may hold lessons on the value of early therapy and immune tolerizing regimens.

AAV treatment and immunosuppression studies in Pompe mice

Immunosuppression with bortezomib and anti-CD20 mAb is effective in reducing neutralizing antibodies to allow repeated AAV administration in mice*.* Su Jin Choi (young investigator awardee) and colleagues at Duke Univ.

Take homes:

- Neutralizing antibodies limit AAV based therapy.
- Antibodies may be pre-existing because of a natural AAV exposure, a cross reactive antibody, or because of a previous AAV vector-based therapy.
- Figuring out how to get around this limitation is important.
- Using the drug bortezomib and a monoclonal antibody (anti-CD20 mAb) researchers treated mice with neutralizing antibodies successfully, and were able to retreat mice with vector using this approach.
- The findings need confirmation and further testing, but if proven safe and effective this treatment could increase gene therapy options for patients.

Neutralizing antibody ablation removes antibodies which interfere with AAV gene therapy

IdeS: An enabling technology to overcome the limitations of neutralizing antibodies to AAV gene therapy. Jeffrey M. Alexander and colleagues at Spark Therapeutics, Inc.

Take homes:

- This is another approach to dealing with neutralizing antibodies.
- The name of the drug is a bit confusing for the MPS community because it is called IdeS, which looks a lot like IDS or Ids, which are common abbreviations for iduronate sulfatase, the enzyme deficient in MPS II.
- Imlifidase (IdeS) is a type of antibody cleaving enzyme.
- Drops in antibodies from IdeS treatment are rapid and transient.
- In mice and primates, this approach overcome neutralizing antibodies.
- In primates it enabled successful vector re-administration.
- This is a promising approach to overcoming neutralizing antibodies.

Other Presentation with Implications for MPS and ML Including Basic Lysosomal Biology

COVID-19/SARS-CoV-2 and lysosomal storage disease patients

Impact of SARS-CoV-2 on patients with lysosomal diseases in a major NYC hospital system. Heather A. Lau at NYU School of Medicine

Take home

- COVID-19 has generated a great deal of concern in the LSD/MPS and ML communities.

- Concerns involved disease exposure and the challenge of continued care including ERT.
- This study queried 139 patients in NYC from March to June 2020 at the height of the first wave.
- The overwhelming majority of these (135) patients had Gaucher disease.
- 30 of the 139 patients were documented to have had COVID-19 including one with MPS IIIA.
- One 55 year old patient on ERT with GD hospitalized and subsequent succumbed.
    - Patient co-morbidities morbid obesity, COPD, hypertension and diabetes.
- All of the remaining COVID-19 affected patients had a mild to moderate COVID19 course.
    - The study was too small to draw statistically valid conclusions.
- In the studied LSD patients COVID-19 course varied from asymptotic to critically severe.
- Risk factors involved baseline health issues, age, and associated co-morbidities.

Basic Cell Biology and Lysosomal Storage Disease

Novel regulatory function of GCN5L1 in lysosomal tubulation and biogenesis. Allen Seylani (young investigator awardee) and colleagues at the NIH

Take home:

- Basic studies of cell biology and lysosomal function may seem far away from treatments for MPS and ML, but they have the power to help us understand clues to lysosome function.
- GCN5L1 is a protein that helps in the dynamic life of a lysosome's formation.
- Disrupting GCN5L1 function in the liver disrupted normal lysosome dynamics.
- This resulting in a dramatic increase in accumulation of autolysosomes.
- Associated with these changes were changes in intracellular signaling and gene expression.
- These types of studies can examine by a sort of reverse engineering, what goes wrong in lysosomal storage diseases.
- This could open up new areas of knowledge about how to treat lysosomal diseases.

ERT combined with a chaperone or substrate reduction drug therapy

Late Onset Pompe Trial

Top Line Results From the PROPEL Phase 3 Study Comparing AT-GAA (cipaglucosidase alfa/miglustat) versus alglucosidase alfa/placebo in Late Onset Pompe Disease. Benedikt Schoser at the Ludwig-Maximilians-Universität München, Munich, Germany.

Take home:

- Some diseases in the LSD group have had approved therapies long enough that researchers are now trying to make improvements.
- This study was testing an Amicus Therapeutics approach to potentiating an enzyme by the addition of a chaperone drug.
- The initial findings are supportive of a therapeutic benefit.
- This approach could theoretically find application to MPS disorders with approved ERT.
- The idea is that a chaperone may improve the life span of the enzyme in the blood and help in penetration of hard to reach tissues.

Gaucher disease type 3 Trial

Venglustat combined with imiglucerase positively affects neurological features and brain connectivity in adults with Gaucher disease type 3. Raphael Schiffmann and clinical partners, and colleagues at Sanofi Genzyme.

Take home:

- Venglustat is an oral brain penetrating selective substrate synthase inhibitor.
- This trial evaluated the drug in type Gaucher disease type 3 combined with ERT.
- Results indicated venglustat brain penetration.
- No serious adverse events were noted and non-neurological disease was stable.
- Substrates were reduced in the CSF were reduced and signs of brain disease were improved.
    - Resting-state functional MRI showed improved brain connectivity.
    - Brain volumes increased in 8 patients with analyzable.
    - Areas of brain volume increase and with increased connectivity had overlap.
    - One patient with undetectable CSF veglustat had brain atrophy and reduced connectivity.
- These are compelling early findings of a substrate reduction-based therapy having a CNS effect.
- While no current substrate reduction therapy exists for an MPS disorder, the current study in GD3 supports the principle of a therapeutic benefit of this approach.

Monitoring ex vivo gene therapy treated stem cells

Analysis of genetically engineered stem cell product and follow up of gene therapy patients through high-throughput single cell technologies. Cristina Baricordi and colleagues at Univ of Calgary and AVROBIO, Inc.

Take home:

- Ex vivo gene therapy combined with hematopoietic stem cell transplantation is a powerful tool and many studies at this WORLD highlighted this approach.
- However, there are many unknowns about how individual cells and cell clones may behave after transplant.
- Tools to track single cells that are the result of such a transplant are critical to ensuring long term therapy, efficacy, and safety.
- AVROBIO, Inc uses the ability to analyze in depth single cells to track cells post-transplant.
- While it doesn't necessarily have a direct impact on therapy it is a hugely powerful tool to keep track of the cells providing the therapy.

Overcoming low numbers: Improved statistically approaches to rare disease trials with limited patients

A survey of statistical study design and analysis methods for rare disease development programs. Miganush Stepanians at PROMETRIKA, LLC.

Take home:

- Statistics may not seem exciting, but good statistical approaches can transform rare disease trials.
- Low patient numbers and the ethical imperative to treat patients and not have a placebo group make traditional experimental and statistical approaches difficult.

- Creative experimental design and an improved approach to measured factors can greatly improve the statistical power of a trial very few patients.
- If one had a coefficient that measured the value and utility of a talk divided by the "sexiness" of a talk, this presentation research talk at this WORLD would have scored as among the most useful.

ML Research Collaborations

The Mucolipidosis Collaborative Research Network (MCRN). Jennifer J. Klein and MCRN member colleagues.

Take homes:

- Similar to the theme above of statistics being a limitation of rare disease research, sometimes the limitation is the number of researchers in a field working on a rare disease.
- There may be too few researchers and spread too far apart, such that collaborations may not easily arise.
    - The purposeful formation of research consortia can be a huge benefit in such situations.
- In 2019 a consortium was formed to address just these barriers.
- The MCRN studies ML II and MPS III that result from mutations in GNPTAB.
- Researchers and advocates from a variety of locations and backgrounds come together from cross disciplinary backgrounds with the aim of accelerating therapy discovery.
- This abstract both served as a spotlight on how these programs can work, and it had the added feature of being coauthored by the Society CEO, Terri Klein, and was first authored by Society member Jenny Klein.
- And on that note, this concludes the recap of WORLD*Symposium*™ 2021.