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CLINICAL GENE THERAPY: THE FUTURE IS NOW
FRIDAY, MARCH 1, 2019 | GRADUATE HOTEL MINNEAPOLIS | 10:30 AM – 2:30 PM

KEYNOTE
James M. Wilson, MD, PhD
Rose H. Weiss Professor and Director, Orphan Disease Center, University of Pennsylvania

PANEL
Patients and caregivers representing several rare disease communities share their thoughts, hopes and concerns regarding gene therapy.

RESEARCH AWARDS
To be considered for this award, trainees must submit a personal statement with their abstract submission on how their research or research path contributes to the understanding and/or treatment of rare diseases. Three posters will be selected to receive this award. The author of each personal statement will receive a $100 gift card.

AGENDA
9:00–10:00 Patient Advocacy Group Breakfast
10:30–12:00 Poster Session
11:45 Lunch
12:00–2:00 Program
2:00–2:30 Networking

RESEARCH POSTER ABSTRACT SUBMISSION:
Research poster abstract submissions will be accepted on basic, translational, and clinical research related to rare diseases and their treatments.

RSVP and Abstract Submissions: https://z.umn.edu/rdd2019
Alphabetical List of Patient Advocacy Groups & Sponsors

**Patient Advocacy Groups**
ALS Association  
Danny’s Dose Alliance, Inc.  
Engage Health, Inc.  
Epilepsy Foundation of Minnesota  
Hemophilia Foundation of MN/Dakotas  
Huntington’s Disease Society of America, MN Chapter  
India Organization for Rare Disorders  
LGS Foundation  
Muscular Dystrophy Association of MN/Dakotas  
National Ataxia Foundation  
Organic Acidemia Association  
Paul and Sheila Wellstone Muscular Dystrophy Center  
Pompe Warriors Foundation  
Prader-Willi Syndrome Association, MN Chapter  
Pulmonary Fibrosis Support Group of Minnesota  
Rare Action Network  
Rein in Sarcoma Foundation  
Tuberous Sclerosis Alliance  
United Mitochondrial Disease Foundation

**Sponsors**
Abeona Therapeutics  
College of Pharmacy, UMN  
Community Engagement to Advance Research and Community Health (CEARCH) Community Health Connections Award, Clinical and Translational Science Institute, UMN  
EveryLife Foundation  
Fairview Pharmacy Services  
Gillette Children’s Specialty Healthcare  
Greenwich Biosciences  
Horizon Pharma  
Orchard Therapeutics  
Sarepta Therapeutics  
Takeda Pharmaceuticals  
Upsher-Smith
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Abstract: Current therapeutic approaches to PAH treatment have been predicated on modification of PVR, but studies to date have shown only modest gains in enhanced functional capacity and mortality. Determination of pulmonary circulation compliance has more tightly correlated with clinically meaningful outcomes including death, functional capacity, and prediction of clinical worsening. We hypothesized that the addition of intravascular compliance via an implanted device would augment pulmonary circulation compliance, reduce pulse pressure (a driver of mechano-transductive adverse remodeling), reduce pulmonary artery elastance, enhance diastolic pulmonary blood flow, and enhance cardiac output at both rest and exercise. The device could be expected to inhibit pulmonary reflectance waves created in the non-compliant, highly bifurcated pulmonary arteries. We designed a passive, implantable pulmonary artery balloon system, driven exclusively by changes in intra-cardiac pressure, to mimic the reservoir function of normal pulmonary arteries. The device was positively evaluated in computer simulation, and subsequently in Brisket disease calves raised in Minneapolis. Durability studies were carried out in an ovine model. The device was then temporally implanted in humans and it functions confirmed at rest and exercise (data to be shown). The device does not function by targeting selected molecular pathways. Rather it is pathway agnostic and modifies final common pathway biophysical events.
Abstract:

Background: Duchenne muscular dystrophy (DMD) is an X-linked recessive genetic disease in which the dystrophin gene is mutated resulting in an absent protein. While the major focus has been placed on the muscle fiber, the vasculature has also been shown to be perturbed in DMD and the DMD model mdx mice. Transcriptome analysis revealed an angiogenic defect related to (Vascular Endothelial Growth Factor) VEGF and its decoy receptor Flt1, in DMD and mdx mice. Interestingly, mdx mice haplosufficient for Flt1 (mdx:Flt1+/-) show increased angiogenesis and improved muscle histology and function.

Objectives: We investigated the cellular mechanism behind the improvement seen in the mdx:Flt1+/- mice in conditional Flt1 knock-out mice using Loxp-Cre technology. We also assessed siRNA, macromolecule inhibitor, commercially available monoclonal antibodies and humanized camelid antibodies against mouse and human Flt1 in development and pre-clinical studies to investigate therapeutic candidates for the treatment of DMD.

Results: Conditional knockout experiments reveal that deletions of endothelial derived Flt1 is sufficient to increase angiogenesis and ameliorate the muscle pathology in mdx mice. Flt1 blockade using several strategies listed above could be systemically delivered in perinatal mdx mice to decrease DMD associated muscle pathology in adult mice. In particular, the camelid antibody could improve muscle force at a clinical viable dose for monoclonal antibodies.

Conclusion: We conclude that anti-Flt1 treatment can improve the muscle phenotype in the mdx mice, validating Flt1 as a therapeutic candidate for the treatment of DMD.
Poster Number: 3  
First Author: Jennifer Triemstra  
Contributing Authors: Michael Privitera¹; Maria Mazurkiewicz-Beldzinska²; Eric Marsh³; Vicente Villanueva⁴; Kevan VanLandingham⁵; Daniel Checketts⁶; Volker Knappertz⁵  
Author Affiliations: 1: University of Cincinnati Medical Center, Cincinnati, OH, USA; 2: Medical University of Poland; 3: The Childrens Hospital of Philadelphia, Philadelphia, PA, USA; 4: University and Polytechnic Hospital La Fe, Valencia, Spain; 5: Greenwich Biosciences, Inc., Carlsbad, CA, USA; 6: GW Research Ltd, Cambridge, UK  
Subject Area: Neurological  
Keywords: Lennox-Gastaut syndrome, Dravet syndrome, cannabidiol, Epidiolex  
Abstract Title: Time to Onset of Efficacy of Cannabidiol (CBD) During Titration in Patients with Lennox-Gastaut Syndrome or Dravet Syndrome Enrolled in Three Randomized Controlled Trials  
Abstract: Add-on CBD significantly reduced seizures associated with Dravet syndrome (DS) or Lennox-Gastaut syndrome (LGS) with an acceptable safety profile in 3 Phase 3 randomized controlled trials in patients with DS (GWPCARE1/NCT02091375) and LGS (GWPCARE3/NCT02224560; GWPCARE4/NCT02224690). A post-hoc analysis of these studies was conducted to determine the time to onset of efficacy by cumulative day during titration. Patients received a plant-derived pharmaceutical formulation of highly purified CBD in oral solution (Epidiolex; 100 mg/mL) at 10 mg/kg/day (GWPCARE3) or 20 mg/kg/day (all trials), or placebo. CBD treatment started at 2.5 mg/kg/day and reached 10 mg/kg/day on Days 7/8 and 20 mg/kg/day by Day 11 in higher dose group. Cumulative frequencies of convulsive (DS) and drop seizures (LGS) were calculated as 28-day averages for each titration day (including previous treatment days). Treatment-emergent adverse events (AEs) were assessed. Overall, 296 patients were randomized to CBD and 220 to placebo. Mean age was 10 years (DS; GWPCARE1) and 15 years (LGS; GWPCARE3/GWPCARE4). In GWPCARE1, nominal statistical significance versus placebo was achieved at Day 10 (P=0.0261) and, in GWPCARE3/GWPCARE4 (pooled data), at Day 6 for the 20 mg/kg/day arm (P=0.0061) and at Day 8 for the 10 mg/kg/day arm (P=0.0368). Of patients with AEs, 60% had their first during titration. Differences for CBD versus placebo in overall AEs and some common AEs were evident during titration but generally to a lesser degree than during the treatment period. Most common (10%) AEs: somnolence, decreased appetite, fatigue, and diarrhoea. Findings suggest that the titration schedule used in the GWPCARE trials led to a significant treatment effect for CBD within 6â€“10 days during up titration.
Abstract: Although commonly misrepresented, CBD does not act directly through cannabinoid receptors at physiologically achievable concentrations. CBD has shown anticonvulsant properties in non-clinical studies and antiseizure effects in clinical trials of Dravet and Lennox-Gastaut syndromes. We present preclinical evidence for a unique multimodal molecular target profile distinct from other antiepileptic drugs. Preclinical evidence suggests CBD reduces neuronal hyperexcitability through multiple mechanisms, including modulation of intracellular calcium via G protein-coupled receptor 55 (GPR55), extracellular calcium influx via transient receptor potential vanilloid type 1 (TRPV1) channels, and adenosine-mediated signalling. CBD antagonises GPR55 at excitatory synapses. Inhibition of intracellular calcium release decreases excitatory currents and seizure activity. GPR55-mediated modulation of neurotransmission was potentiated in excitatory neurons and reduced in inhibitory neurons in a chronic epilepsy model. CBD potently blocked GPR55-mediated increase of miniature excitatory postsynaptic current frequency in pyramidal neurons in both healthy and epileptic tissue. CBD did not affect GPR55-mediated increase of excitatory neurotransmission in inhibitory neurons in healthy tissue. CBDs anticonvulsant properties were attenuated in GPR55 knockout (KO) animals. CBD desensitises TRPV1 channels. The resultant decrease in extracellular calcium influx decreases neurotransmission. The dose-dependent, CBD-mediated increase in seizure threshold seen in wild-type mice was significantly attenuated in TRPV1 KO mice. Brain CBD concentrations were consistent with those required for TRPV1 activation and desensitisation irrespective of genotype. CBD inhibits the equilibrative nucleoside transporter 1 (ENT1), reducing adenosine reuptake. The increase in extracellular adenosine reduces hyperexcitability and neurotransmission. CBD inhibited [3H] adenosine uptake into rat cortical synaptosomes at low micromolar concentrations. While the precise mechanisms by which CBD exerts its anticonvulsant properties in humans remain unknown, growing preclinical evidence suggests CBD reduces neuronal hyperexcitability through a unique multimodal mechanism of action. CBD antagonizes GPR55 at excitatory synapses, desensitises TRPV1 channels, and inhibits adenosine reuptake.

Funding: GW Research Ltd, Cambridge, UK
Abstract: Background: Gangliosidosis diseases are disorders caused by single enzyme deficiencies that result in ganglioside accumulation throughout the body, with the CNS being the most prominently affected tissue. Phenotypes of these diseases are defined by age of symptom onset: infantile (12 months), late-infantile (12-24 months), juvenile (>24 months) and late-onset (18 years). There are currently no approved treatments for the gangliosidoses. Aside from the challenge of developing therapies that cross the blood brain barrier (BBB), the major hurdle to treatment development is lack of adequate natural history data. If effective treatments were available, understanding genotype-phenotype correlations would be of paramount importance towards determining timing of treatment initiation. This becomes especially important in an era in which newborn screening is becoming available for an increasing number of lysosomal diseases.

Hypothesis/Objectives: The objective of this study is to evaluate the phenotypes associated with the genotypes of patients with gangliosidoses diseases, with specific focus on initial symptoms leading to diagnosis. Methods: This project evaluated clinical changes in patients enrolled in The Natural History of Gangliosidoses study at the University of Minnesota, and looked for patterns of clinical progression and distinguishing features of infantile, late-infantile, juvenile and late-onset disease in both GM1- and GM2-gangliosidosis.
Subject Area: Metabolic, Kidney, Lysosomal Storage Disorder  
Keywords: Children, Genetic renal disease, chronic kidney disease, Cystinosis, Cysteamine  
Abstract Title: Delayed-Release Cysteamine Bitartrate (DR Cysteamine) Controls WBC Cystine Levels and Promotes Growth in Treatment-Naïve Patients <6 Years of Age With Nephropathic Cystinosis

Abstract:

Nephropathic Cystinosis (NC) is a recessive disease in which the lysosomal cystine exporter is deficient, intralysosomal cystine accumulates, and the biomarker, WBC ½ cystine/mg protein is > 1 nmol. Cysteamine bitartrate (Cys-Bi), is used to lower levels to <1 nmol. Previous trials of RP-103 have converted patients from an every 6h form of Cys-bi to this every 12h formulation. We conducted a long-term, prospective, controlled, open-label study of RP-103 in 17 Cys-Bi naïve patients with NC in the US & Brazil. The RP103 starting dose was gradually escalated (10%, every 2 weeks) until the WBC ½ cystine level was <1 nmol. We evaluated height and weight at each visit, and safety (incidence of TEAEs and SAEs). Data reported (mean±) below will be for 15/17 subjects < 6y of age. Age was 2.2±0.9y, range 1.04±4.53y. Prior to RP-103, the WBC cystine concentration was 3.1± 2.9 nmol ½ cystine/mg protein. At each subsequent time, the mean concentration was < than at Day 1: 2.2±3, Week(wk) 2; 1.1±1.3 wk 12; 0.7±0.64, Month(m) 18. By end of study, 76.9% of subjects had a WBC cysteine <1nmol. On Day 1, standing height was in the 2.59±4.00 of the reference population, and increased to 3.27±5.69 at wk 2, 6.95±10.88 at wk 12, and 55.36±43.88 at m18. Z-scores for height were 3.16±1.55 at Day 1 and increased to 0.11±1.96 at study end. On Day 1, weight was in the 3.46±11.13 and increased at study exit to 32.85±35.58. All 17 subjects in the Safety Population had at least 1 TEAE, and 8 had at least 1 TEAE considered related to the study drug. Twelve had an SAE, 5 (29.4%) had a TEAE Grade 3, and 1 (5.9%) died (unrelated to study drug). No TEAEs led to permanent discontinuation of study drug. RP-103 safely and effectively lowered the biomarker to target (<1 nmol) in young Cys-Bi naïve patients with NC. Linear Growth and body mass increased into the normal range for age (z-score) for those 6y. Thus, Cys-Bi naïve patients 6y with nephropathic cystinosis may safely and efficiently be started on cystine reduction therapy with RP-103 (Procysbi).
Urea cycle disorders (UCDs) are rare inborn errors of urea synthesis, characterized by hyperammonemia (HA) and life-threatening hyperammonemic crises (HAC). American and European consensus statements on the diagnosis and treatment of UCDs were last published in 2001 and 2012, respectively; however, due to the rarity of UCDs, recommendations are based primarily on case reports and expert opinion, and there is limited agreement or consistency related to long-term management approaches. A clinician survey was conducted to assess current real-world practices and perspectives on the challenges and unmet needs in UCD care. Here we summarize attitudes related to UCD management and treatment considerations. A survey was administered at the American College of Medical Genetics meeting in 2017, and distributed online from May to September 2017.

Results: Sixty-six US clinicians completed the survey (60 geneticists; 5 biochemical geneticists; 1 pediatric neurologist). Respondents most frequently ranked the top indicators of successful UCD management as absence of HAC (29%), normal neurocognitive function (20%), and optimal quality of life (QOL; 14%). More than 80% of respondents agreed that asymptomatic UCD patients are at risk of brain damage over time due to mild/subclinical elevations in ammonia. The vast majority also agreed that asking UCD patients about a wide range of possible HA symptoms (84%) and tracking less severe symptoms (86%) are routine aspects of their practice. Though >90% of respondents agreed that even modest elevations in ammonia can cause brain damage, and that there is a need for tight ammonia control in asymptomatic and late-onset patients, only 28% indicated that they would aim for normal ammonia if routine, accurate ammonia testing was feasible. Respondents were split between agreement (38%), neutrality (33%), and disagreement (30%) with the concept that low-protein diet and/or amino acid supplements generally offer asymptomatic patients adequate protection from the consequences of elevated ammonia. While one-quarter of respondents were neutral, the remainder of the sample was divided between agreement/disagreement with the notions that long-term nitrogen scavenging therapy is generally warranted only for patients with history of HAC, and that all long-term nitrogen scavengers are equally effective; however, >90% agreed that patients have better disease control when they are more adherent to their UCD therapy. Nearly 90% indicated that clinicians and patients would benefit from updated UCD management guidance. In 2017, clinicians who treat patients with UCDs are most commonly focused on preventing HAC, maintaining neurocognitive function, and optimizing QOL. The vast majority reported concern about the potential neurologic consequences of mild or subclinical HA, and typically ask patients about a wide range of potential manifestations of HA, including less severe symptoms, as part of routine practice. This survey suggests a need for updated expert guidance on the long-term treatment and management of asymptomatic UCD patients.
Abstract: Introduction: Alpha-mannosidosis is a rare lysosomal storage disease with multisystemic abnormalities and a wide clinical variability. The primary clinical brain MRI findings are described in case report studies. Quantitative MRI outcomes using robust automated methods as a prerequisite for clinical trials are not available.

Methods: High-resolution T1-weighted MRI scans of 6 subjects (age: range 6-27; mean ± SD 14.5±4.9 years of age) with alpha-mannosidosis acquired on clinical MRI scanners were evaluated by automated volumetric analysis and compared to MRIs of 80 healthy controls (age: range 6-25; mean ± SD 14.0±7.7 years of age). Brain volumes were adjusted for intracranial volume and age.

Results: Lower volumes of total cerebral grey matter (specifically putamen*, caudate**, and thalamus*) and cerebellum* (both white* and grey* matter) were observed in patients. No change was seen in volumes of cerebral cortex, corpus callosum, total cerebral white matter, and brainstem. (*p<0.001; **p<0.02)

Conclusions: In the cohort of 6 alpha-mannosidosis patients brain MRI atrophy of subcortical grey matter structures and cerebellum and a preservation of cerebral white matter structures were quantified by automated volumetric analysis. These findings will be later assessed to determine correlation to functional motor and cognitive scores.
Discovery of brain MRI signatures in infants with severe form of MPS I in the pre-HSCT and post-HSCT stages

Introduction: Hurler syndrome (MPS IH), the most severe form of mucopolysaccharidosis (MPS) type I, is fatal within the first decade of life if untreated. Hematopoietic stem cell transplantation (HSCT) is the standard of care for MPS IH to treat central nervous system disease. As new treatments are being developed and newborn screening identifies patients earlier, it is critical to define reliable and sensitive measures reflecting MPS I disease prior to HSCT in infants.

Methods: MRI scans including T1, T2-weighted images and diffusion tensor imaging (DTI) were acquired on clinical 3T MR system in 12 pre-HSCT MPSIH infants (age < 2 years of age). Brain and ventricular volumes, T1/T2 ratios, and DTI indices were evaluated. Results: Significant increase in fractional anisotropy (FA) between scans prior/after-HSCT and limited FA change in the post-HSCT follow-up scans were seen in most patients. Two patients exhibited no or minimal FA increase between pre-/after-HSCT scans but steeper FA increase on post-HSCT scans. RD and T1/T2 ratio exhibited similar trends. Total brain volumes increased in most subjects after the HSCT; however, ventricular volumes exhibited both increases and decreases within the population. Conclusions: The finding is suggestive of an immediate response measured by DTI and T1/T2 ratio (within months) to the HSCT procedure in most subjects. In some subjects a delayed response (after a year) to the HSCT procedure was detected. The results confirmed an increase in the white matter myelination in patients who received HSCT. Determinants of the response type to the HSCT need to be identified. Similar trends are observed in the normal brain development; thus, the exact trajectory and rate of FA/RD and T1/T2 ratio changes need to be compared with healthy age- and gender-matched individuals.

(Funded by NIH U54NS065768, Lysosomal Disease Network; Orphan Disease Center at the University of Pennsylvania MPSI-16-003-02; the Genzyme Sanofi)
Hurler syndrome is a rare inherited lysosomal storage disorder that, untreated, results in rapid neurocognitive decline after 2 years, multisystem dysfunction, and death before 10 years. Earlier treatment with hematopoietic cell transplantation (HCT), in combination with enzyme replacement therapy (ERT), is associated with better physical and neurocognitive outcomes. As such, Hurler syndrome was added to the Recommended Uniform Screening Panel for newborn screening in 2016. While too soon to assess the impact of early identification and treatment, this case series examines neurocognitive and adaptive functioning in three children identified at birth due to an older affected sibling.

Participants and Methods: Three children (Patients A, B, and C) diagnosed with HS at < 1 month of life underwent early initiation of ERT (at 28, 28, and 7 days, respectively) and HCT (at 8.9 months, 5.0 months, and 4.9 months, respectively). We compare pre-HCT IQ and adaptive functioning to that at most recent follow-up. Upon first evaluation at 3-7 months, all three patients demonstrated average IQ (A=105, B=96, and C=95). At most recent follow-up (6, 5, and 2 years, respectively), all patients maintained average IQs (IQ point change: +0, -3, -10, respectively). Prior to HCT, Patients A and C showed average overall adaptive functioning; Patient B was below average. At most recent follow-up, all three patients were average in overall adaptive functioning and for all subdomains. Maintenance of IQ and adaptive functioning in these infants, identified and treated with ERT at < 1 month old and by HCT at < 9 months old differs from published data in children who started identical treatment at a later age and deteriorated to less than average before stabilization following HCT. This case series provides quantitative evidence for newborn screening and early treatment as key to positive neurocognitive and functional outcomes.
The discovery that patients with Gaucher Disease (GD), a rare lysosomal storage disorder, were developing symptoms similar to Parkinson Disease (PD) led to investigation of the relationship between the two seemingly unrelated pathologies. GD is the result of a biallelic mutation in the gene GBA1, which encodes for the enzyme glucocerebrosidase (GCase). Recently, GBA1 mutations have become recognized as the most common genetic risk factor for PD development. Although the exact mechanism is unknown, current understanding suggests impaired GCase inhibits lysosomal activity and decreases the rate of protein degradation. This can lead to abnormal accumulation and aggregation of the protein α-synuclein, an important component of PD development. Better understanding of how mutated GCase increases risk for α-synuclein pathology could assist with the development of early clinical biomarkers of PD and other synucleinopathies, as well as novel treatments. Historically, α-synuclein has not shown to be a consistent biomarker for PD. However, recent research investigating α-synuclein content within exosomes, small vesicles released by cells, has yielded promising results. In the presence of a GBA1 mutation, the decrease in GCase activity lowers overall lysosome function, increasing secretion of exosomes and potentially altering the contents. We hypothesize that compared to healthy controls, exosomes from patients with PD and/or GD will show significant differences in exosome amounts and contents. Our objective in this proof of concept study is to explore the utility of exosomes as biomarkers and drug targets in these patients and gain insights on the mechanisms linking GCase and PD development. We have adapted a method for isolating plasma exosomes using size exclusion chromatography followed by exosome precipitation. We will adopt this technique to isolate exosomes in patients with PD with and without GBA1 mutation. We will then analyze and compare exosomal protein and RNA content using sensitive immunoassays and sequencing techniques.
Abstract: Type 1 Gaucher Disease (GD1) is a rare genetic mutation of the GBA1 gene, causing the accumulation of glucocerebroside (GC), resulting in inflammation. Compliment 5a and its receptor is a known inflammatory mediator and has been found to be elevated in those with GD1 who are not undergoing treatment. Patients with GD1 either undergo enzyme replacement therapy (ERT) or substrate reduction therapy (SRT) to help alleviate GC within cells. 3 patients receiving ERT, 3 patients receiving SRT, 2 treatment naïve, and 3 controls were all analyzed using the ThermoFisher C5a Human ELISA kit. 3 samples had been collected for each patient over a span of 3 months, allowing for an un-obstructed baseline to be obtained. The C5a concentrations for each group of patients were then analyzed and compared using a 1-way ANOVA test, which showed no significant difference between any of the treatment groups and the control patients. Both ERT and SRT groups were found to have elevated C5a levels compared to the healthy control, but not enough to be significant. The treatment naïve group was found to be lower than that for the healthy controls. Both of these findings warrant further tests with larger sample sizes before any definite conclusions can be made.
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Subject Area: Hematological; Cancer
Keywords: Fanconi Anemia, Cancer, inflammation
Abstract Title: Suppression of cell debris-stimulated Fanconi Anemia cancer progression by resolvin mediated clearance
Abstract: While current clinical evidence links Fanconi Anemia (FA) to an increased risk of aggressive blood, head, and neck cancers, the mechanisms underlying this link remain poorly understood. Furthermore, Fanconi Anemia patients experience increased mortality and tumor recurrence rates in response to cytotoxic cancer treatments, such as chemotherapy and radiation. While these treatments reduce tumor burden by killing tumor cells, the resulting dying tumor cells, or cell debris, induces an inflammatory response within the tumor microenvironment that in turn stimulates the growth of surviving tumor cells and contributes to tumor relapse. Thus, there exists an urgent need for novel anti-cancer agents in these patients. Specialized pro-resolving mediators (SPMs) are endogenous lipid autacoids derived from omega-3 polyunsaturated fatty acids that mediate the termination of inflammation and promote a return to tissue homeostasis. SPMs such as resolvins act by enhancing macrophage clearance of inflammatory debris and associated inflammation, without being immunosuppressive. Thus, resolvins may represent a novel adjuvant treatment modality in Fanconi Anemia-related cancers, via stimulating macrophage clearance of therapy-generated tumor cell debris and inflammation, thereby reducing tumor recurrence and therapy-related mortality. Here, we demonstrate that FANCC\textsuperscript{-/-} tumor cells exhibit an increased inflammatory response to chemotherapy compared to wild type (WT) tumor cells, as quantified by cytokine/chemokine expression profiles. We further demonstrate that FANCC\textsuperscript{-/-} tumors exhibit accelerated growth in vivo compared to WT tumors. Resolvins were found to suppress both WT and FANCC\textsuperscript{-/-} tumor growth in vivo and did not affect tumor cell growth in vitro, suggesting a stromal-dependent anti-tumor mechanism. Resolvins further stimulated murine macrophage phagocytosis of chemotherapy induced WT and FANCC\textsuperscript{-/-} head & neck tumor debris in vitro. Thus, our results establish the therapeutic enhancement of endogenous resolution via resolvins as a novel modality to complement current cytotoxic cancer treatments in Fanconi Anemia patients, and highlights inflammation as a potential link between Fanconi Anemia and increased rates of post-therapeutic cancer mortality and recurrence.
Pain is one of the major comorbidities of sickle cell disease (SCD) requiring chronic opioid therapy (COT). COT has been associated with reduced survival and opioid-induced hyperalgesia (OIH). To examine the effect of COT on pain and survival in SCD, we performed a randomized double-blind placebo-controlled trial using female homozygous HbSS BERK (sickle) and HbAA BERK (control) mice. Mice were injected subcutaneously daily with either morphine sulfate at a starting dose of 20 mg/kg, which was increased by 5 mg/kg after weeks 12, 18, 28, 30, and 38 respectively or equal volume of saline until the end of survival. We observed significantly decreased survival of saline-treated sickle mice compared to saline-treated control mice (P = 0.0009). Compared to saline, morphine treatment led to a significant decrease in survival in control mice (P = 0.035) but not in sickle mice (P > 0.05). We did not observe an association between hyperalgesia and survival in either control or sickle mice. However, we discerned a significant increase in mechanical, cold, and heat hyperalgesia in control mice after 4 weeks of morphine treatment (P < 0.02, compared to day 0) which continued to increase up to 12 weeks (P < 0.05 compared to week 4). Similarly, in sickle mice we observed an increase in mechanical hyperalgesia after 4 weeks (P < 0.02 compared to day 0) of morphine treatment which continued to increase up to 12 weeks (P < 0.0001).

These data suggest COT leads to OIH in both control and sickle mice. Morphine treatment continued to show an analgesic effect over the course of 12 weeks at a constant dose. Thus, mice did not develop tolerance to morphine analgesia. COT lead to OIH in sickle mice, but provided analgesia without causing tolerance or reducing survival.
Poster Number: 15
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Subject Area: Metabolic
Keywords: Genetic engineering, cell-based enzyme replacement therapy, IDUA, MPS I
Abstract Title: CRISPR/Cas9 mediated insertion of α-L-iduronidase in B cells for sustainable IDUA expression for treating mouse model of MPS I

Abstract: Mucopolysaccharidosis type I is a rare inherited lysosomal storage disorder caused by mutations in the IDUA gene. IDUA deficiency results in the accumulation of glycosaminoglycans (GAGs), which ranges in clinical severity from Scheie syndrome (mild form) to Hurler syndrome (Severe form). At present, enzyme replacement therapy (ERT) and bone marrow transplantation (BMT) are the standard of care for this disease with various effectiveness. CRISPR/Cas9 open new avenues for genetic engineering of primary human cells. CRISPR/Cas9 have been demonstrated to efficiently engineered immune cells1-3. B cells are the backbone of the humoral immune system that can be activated into long-lived plasma cells, capable of generating and secreting high level of protein (Antibodies). Here we describe a system to assessed B cells for cell-based enzyme replacement therapy. B cells were engineered using CRISPR/Cas9 system, subsequent in up to 90% editing efficiency. Next, B cells are genetically engineered with a combination of CRISPR/Cas9 reagents and AAV containing eGFP DNA template flanked with homology arms of up- and downstream of a target site. Up to 50% of total B cells were eGFP positive at 12-day post-engineering. Furthermore, the systems were compared between the system that expression of the eGFP relies on endogenous cis-promoter of the target site and the system where the eGFP relies on a synthetic MND promoter. The expression of eGFP under MND promoter showed over 100x fold greater GFP expression (measured by MFI) than of eGFP under endogenous cis-promoter. In summary, B cells can be efficiently genetic engineered to stably express a protein of interest (eGFP) using a combination of CRISPR/Cas9 and AAV systems. Moving forward, we will utilize this method to engineer B cells to express IDUA enzyme as a cell-based enzyme replacement therapy for treating IDUA deficient mouse model.
Abstract: Background: Hypophosphatasia (HPP) is a rare bone mineralization disorder caused by loss-of-function mutations in the ALPL gene encoding tissue nonspecific alkaline phosphatase (TNAP). Physical effects of the disease, including as soft or weak bones, premature loss of teeth, skeletal abnormalities and impaired mobility, are well-known. However, sparse literature is available regarding the neuropsychological manifestations of HPP, especially in children. HPP could impact neurocognitive development and emotional health via several processes. Alterations in myelin formation and synaptic plasticity due to the loss function of TNAP, lack of availability of vitamin B6 for production of neurotransmitters, the presentation of epileptic encephalopathy, and chronic disease burden are potential mechanisms that could result in neurocognitive abnormality and/or mood effects in some patients. Methods: The present study aimed to investigate risk for behavioral health challenges in children with HPP. Parents of affected children (n=26) completed surveys offering insight into their childs adaptive function, social-emotional well-being, and psychopathological comorbidity. Results: According to parent ratings, approximately 1 in 3 children with HPP exhibited moderate to severe symptoms of hyperactivity, aggression, depression, somatization, and/or attention problems. Adaptive functioning for 28% of the study cohort was rated as below average or impaired. Responses on a symptom checklist for attention deficit hyperactivity disorder (ADHD) showed that, out of the 19 children age 5 or older, 53% of participants had sufficient parent-rated symptoms to meet diagnostic criteria for some form of ADHD. Interpretation: These findings suggest that children with HPP are at heightened risk for ADHD symptoms and may struggle with emotional and adaptive functioning at a higher rate than their unaffected peers. The current lack of information on social, emotional and behavioral functioning in HPP limits the ability of practitioners to effectively address mental health-related issues, and also misses an important opportunity to measure potential effects of therapy.
Patients with Rett syndrome (RTT) manifest abnormal cutaneous sensitivity and apparent diminished pain response. The nature of their sensory abnormalities is not well understood and characterizing somatosensory mechanisms is difficult in nonverbal populations. We tested the feasibility of using baseline heart rate variability (HRV) to predict behavioral reactivity during a standardized modified quantitative sensory test (N=15). Results indicate that baseline HRV was low and predicted behavioral reactivity to cool and mechanical, but not other stimuli. These preliminary findings provide feasibility evidence for our approach and are consistent with preclinical evidence of mechanical and cold hypersensitivity and heat hyposensitivity.
Abstract: Glioblastoma (GBM) is an aggressive and infiltrative primary brain tumor with a median survival of 14.6 months following the current treatment strategy of radiation and chemotherapy. Therefore, there is a need to develop strategies to enhance the efficacy of chemo-radiation treatments for GBM. DNA damage response signaling pathways play a critical role in DNA repair and cell survival following radiation therapy and the inhibition of these pathways could augment the cytotoxicity associated with radiation providing a radiosensitizing effect. Ataxia Telangiectasia and Rad3-Related Protein (ATR) is a key regulator of the DNA damage response network and VX-970 is the first potent and selective inhibitor of ATR to enter clinical trials. Preliminary in vitro studies from our lab to determine a dose dependent effect of VX-970 in combination with a radiation dose of 5 Gy on the cell survival indicated that administration of radiation led to an enhancement in the cell death with an increasing dose of VX-970 in the U251 human GBM cell line. We evaluated the BBB penetration of VX-970 and studied the role of efflux transporters on the brain exposure of VX-970 in preparation for efficacy studies in PDX models of GBM. Brain distribution studies were performed in wild-type and Mdr1a/b-/ Bcrp1-/- (triple knockout) FVB mice (n=4) following intravenous administration of 20 mg/kg VX-970. Plasma and brain samples were collected at 7 time points post dosing and were analyzed using LC-MS. The brain-to-plasma (B/P) ratio in transporter (Pgp and Bcrp) knockout mice was 17.5 as opposed to 0.8 in transporter intact wild-type mice. This 22-fold increase in the B/P ratio in the triple knockout mice indicates that Pgp and/or Bcrp play a significant role in the efflux of VX-970 from the brain thereby limiting its brain penetration. Oral administration of VX-970 at a dose of 20mg/kg in transporter intact wild-type mice indicated an oral bioavailability of 38%. Steady state brain distribution studies were also performed in wild-type and triple knockout FVB mice following intraperitoneal implantation of the Alzet osmotic pumps to release VX-970 at a rate of 10ul/hr for 48 hours. We observed that the B/P ratio in transporter (Pgp and Bcrp) knockout mice was 12.6 as opposed to 0.12 in transporter intact wild-type mice. We are in the process of conducting additional studies in Pgp and Bcrp single knockout mice to determine a more precise status of the role of efflux transporters in the brain delivery of VX-970. The free fraction of VX-970 was found to be 6.1% in plasma indicating that it has a relatively high unbound fraction as compared to other chemotherapeutics for brain delivery. We are in the process of determining free fraction in the brain and relating the concentration of free drug to a dose range associated with effective radiosensitization through efficacy studies in the PDX models of GBM. This PK-PD relationship will help guide future clinical trials.
Poster Number: 19
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Subject Area: Neurological
Keywords: Morquio syndrome (MPS IV), mucopolysaccharidosis (MPS), neuropsychology, questionnaire, quality of life (QOL)
Abstract Title: Emotional, social, behavioral, and pain self-report measures and outcomes in Morquio syndrome

Abstract:
Mucopolysaccharidosis IV, Morquio syndrome (MPS IV), is an autosomal recessive disorder caused by the deficiency of N-acetylgalactosamine-6-sulfate sulfatase, resulting in wide clinical heterogeneity characterized by significant skeletal manifestations (Peracha, 2018) and severe short stature. In the literature, there is minimal information regarding behavior, emotional, and social outcomes in MPS IV; however, one previous study has demonstrated score elevation on scales assessing anxiety, depression, and withdrawal (Davison, 2013). The objective of this cross-sectional study is to explore the behavioral, emotional, social, and pain outcomes of MPS IV individuals utilizing the NIH-developed PROMIS questionnaires. MPS IV participants who were enrolled in a multicenter study of brain structure and function in MPS disorders (U54NS065768) were administered the questionnaires to assess anger, anxiety, relationships, support, fatigue, and pain. Eight subjects completed the questionnaires (6 females, 2 males). Half were adult (N > 18yo = 4) and half were pediatric (N < 18yo = 4) with an age range of 11-53. A standardized T-score of 50 (±1 SD=10) is the average score for the general population. Adults and pediatrics were given similar assessments respective of age. In both groups, no average T-scores were ±1 SD or greater for any domains. For pediatric subjects, average T-scores >±5 of 50 was found only for the fatigue scale (T-score: 55.525, range: 45.4-63.6). For adults, average T-scores >±5 of 50 were found for anxiety (T-score: 56.0, range: 50.8-60.7), pain interference (T-score: 56.6, range: 40.7-63.5), emotional support (T-score: 55.75, range: 52.1-63.5), and informational support (T-score: 57.875, range: 48.9-69.1). Pediatrics and adults scored similarly on questionnaires relating to pain quality and peer relationships/isolation. This pilot data suggests average outcomes for MPS IV in areas described above. However, several caveats—"including small sample size and lack of control data (for example, administration of PROMIS to other MPS types)—merit further inquiry.
Spinocerebellar ataxia type 1 (SCA1) is a fatal neurodegenerative disease caused by an abnormal expansion of CAG repeats in the Ataxin-1 (ATXN1) gene. SCA1 is characterized by motor deficits and cerebellar Purkinje cell neurodegeneration. Even though mutant ATXN1 is expressed from an early age, disease onset usually occurs in one's thirties, indicating the presence of compensatory factors that limit the toxic effects of mutant ATXN1 early in disease. Brain derived neurotrophic factor (BDNF) is a growth factor known to be important for the survival and function of cerebellar neurons. Here, we characterized cerebellar BDNF expression levels during disease progression and examined the therapeutic potential of BDNF delivery via osmotic ALZET pumps in a SCA1 transgenic mouse model. Our results indicate that BDNF expression varies with disease progression. Also, delivery of extrinsic BDNF during early disease progression delays onset of motor deficits and some neuronal pathology in a ATXN[82Q] mouse model.
Mutations in sorting nexin 10 (Snx10) have recently been found to account for roughly 4% of all human malignant osteopetrosis, some of them fatal. To study the disease pathogenesis, we investigated the expression of Snx10 and created mouse models in which Snx10 was knocked down globally or knocked out in osteoclasts. Endocytosis is severely defective in Snx10-deficient osteoclasts, as is extracellular acidification, ruffled border formation, and bone resorption. We also discovered that Snx10 is highly expressed in stomach epithelium, with mutations leading to high stomach pH and low calcium solubilization. Global Snx10-deficiency in mice results in a combined phenotype: osteopetrosis (due to osteoclast defect) and rickets (due to high stomach pH and low calcium availability, resulting in impaired bone mineralization). Osteopetrorickets, the paradoxical association of insufficient mineralization in the context of a positive total body calcium balance, is thought to occur due to the inability of the osteoclasts to maintain normal calcium phosphorus homeostasis. However, osteoclast-specific Snx10 knockout had no effect on calcium balance, and therefore led to severe osteopetrosis without rickets. Moreover, supplementation with calcium gluconate rescued mice from the rachitic phenotype and dramatically extended life span in global Snx10-deficient mice, suggesting that this may be a life-saving component of the clinical approach to Snx10-dependent human osteopetrosis that has previously gone unrecognized. We conclude that tissue-specific effects of Snx10 mutation need to be considered in clinical approaches to this disease entity. Reliance solely on hematopoietic stem cell transplantation can leave hypocalcemia uncorrected with sometimes fatal consequences. These studies established an essential role for Snx10 in bone homeostasis and underscore the importance of gastric acidification in calcium uptake.
Morquio syndrome, mucopolysaccharidosis type IVA (MPS IVA), is caused by deficiency of the degradative lysosomal enzyme N-acetylgalactosamine-6-sulfatase, leading to cellular glycosaminoglycan deposition and progressive, severe skeletal dysplasia, dental abnormalities, corneal clouding, and severe short stature. Morquio syndrome has not typically been associated with neurocognitive deficits but a recent publication suggests evidence of problems with attention and behavioral control in the MPS IVA population (J Inherit Metab Dis (2013) 36:323-328). The objective of this cross-sectional study was to investigate potential neurocognitive abnormalities in MPS IVA via direct measurements of neuropsychological functioning. MPS IVA participants from a multicenter study of brain structure and function in MPS disorders were administered a comprehensive neuropsychological assessment of intellectual functioning, attention, verbal and visual memory, visual-spatial skills, and adaptive functioning. Test performance yields standardized scores with $\mu=100$ and $SD=15$, with qualitative ranges of average (85-115), below average (70-84), and impaired (<70). The study cohort (N=11) consisted of approximately equal pediatric and adult participants ($N<18yo=6$; $N\geq18yo=5$) with a median age of 17 (range 11-53). Results indicated average functioning across all domains except attention. All ages were below average on a scale related to consistency of focus, with mean standard scores of 71 for pediatrics (SD 27; median 73; range 40-106) and 78 for adults (SD 12; median 76; range 64-92). In addition, pediatric participants were below average for sustained attentional vigilance, with a mean standard score of 72 (SD 36; median 68; range 40-110). These observations offer quantifiable evidence of attention problems in patients with Morquio syndrome. Findings should be considered in the context of mounting evidence that neurocognitive problems exist even in MPS types with normal IQs, such as MPS I and MPS II. National MPS Society, Lysosomal Disease Network U54NS065768, UMN Center for Neurobehavioral Development.
Poster Number: 23
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Subject Area: Neurological
Keywords: intramuscular intravenous allopregnanolone status epilepticus dogs
Abstract Title: Intramuscular versus Intravenous Allopregnanolone Pharmacokinetics and Safety in Dogs for Early Treatment of Status Epilepticus
Abstract: Allopregnanolone (ALLO) is a neurosteroid that positively modulates synaptic and extrasynaptic GABA-A receptors. Given its physicochemical and pharmacological properties, we hypothesize that ALLO may be useful as a first-line treatment for both human and canine status epilepticus (SE). Our objectives were: 1) compare the pharmacokinetics (PK) of intramuscular (IM) and intravenous (IV) ALLO including estimation of IM bioavailability, 2) assess the IM and IV adverse effects, and 3) simulate dosing regimens that would provide at least 50% seizure protection for a minimum of 15 minutes following the start of an IV infusion or immediately after an IM injection. Our subjects included healthy dogs (n=3) and those with spontaneous epilepsy (n=2, 1 on phenobarbital [PB]). Single ALLO doses ranging from 1-6 mg/kg were infused IV over 5 minutes or administered IM. Serial blood samples were collected up to 6 hrs post-dose. Plasma ALLO concentrations were measured by a UPLC-MS/MS system. Non-compartmental and compartmental PK analyses were performed. Concentration-time profiles were best fit by a two-compartment model (IV t1/2: alpha: 1.6-3.1 min, beta: 13-38 min; IM t1/2alpha: 1.9 min, beta: 158 min). IM bioavailability was estimated to be 70%. There was a dose-dependent increase in ataxia and sedation. Peak effects occurred 3 minutes into the infusion and 3-5 following IM injection, with a return to baseline within 20 minutes. Simulations suggest that a 6 mg/kg IM or 2.5 mg/kg IV dose can attain concentrations associated with 75% seizure protection in mice. In conclusion, IM ALLO PK differs from its PK following IV administration with the former exhibiting a longer beta t1/2, and intermediate bioavailability. IV ALLO exhibited a rapid central nervous system effect. IM and IV doses up to 2 mg/kg were well-tolerated in dogs. Our results support further evaluation of ALLO for SE in dogs and humans.
Abstract: One of the most prevalent features in neurodegenerative diseases is mitochondrial abnormalities. Indeed, it has been reported that all patients with frontotemporal dementia (FTD) amyotrophic lateral sclerosis (ALS) and have mitochondrial dysfunction. Recently, several mutations of CHCHD10 encoding a mitochondrial protein were reported as a genetic cause of ALS and FTD. Although mutations in over 20 genes have been identified as the cause of ALS and FTD, only CHCHD10 primarily localizes in mitochondria. This suggests that mitochondrial defects might be one of the primary causes of neurodegeneration in ALS and FTD patients and that understanding of the CHCHD10-mediated mitochondrial pathogenesis would be helpful to understand mitochondrial dysfunction that is shared in all ALS and FTD patients but not fully understood. In addition, it will also be helpful to develop therapeutic strategies that can be applied to all patients regardless of their genetic causes. To understand the disease-causing mechanism of CHCHD10, we generated a fruit fly (Drosophila) model. When a mutant form of CHCHD10 was expressed in Drosophila eyes, motor neurons and muscles respectively, CHCHD10-mutantit caused corresponding degenerative phenotypes. Furthermore, mutant CHCHD10 overexpression in mammalian cells caused mutant-dependent mitochondrial fragmentation. To further investigate mitochondrial pathways important for CHCHD10-mediated pathogenesis, we performed a forward genetic screening against mitochondrial proteins with the Drosophila model and transgenic RNAi flies. Knockdown of two Parkinsons disease-causing genes (PINK1 and Parkin) significantly rescued the degenerative phenotypes. We are currently investigating how the PINK1/Parkin pathway contributes to the pathogenesis of mutant CHCHD10-induced ALS and FTD and whether it can be a therapeutic target.
Abstract:

Background: Immune responses to gene therapy have been recognized as a threat to efficacy and safety since the early days of gene therapy research. As the number of gene therapies moving into clinical trials increases, immune responses that can occur towards the vector vehicle, the transgene, or both continue to be of primary concern. Several gene therapy clinical trials have employed immunosuppressive regimens to tolerize the patient to the gene therapy in order to maximize efficacy and minimize the toxicity of the gene therapy. But the immunosuppressive agents themselves also impose toxicities, including both short-term and long-term risks. The optimal immunosuppressive regimen should result in immune tolerance and a sustained and therapeutic level of gene expression, while imposing minimal number of adverse effects. Objectives: The objectives of this study are to provide a review of specific immunosuppressive regimens that have been used in gene therapy clinical trials, describe their efficacy and toxicity, as well as provide an overview of immunosuppressive agents. Mechanisms of action, specific immune system targets, and toxicities are described. Although targeted immunosuppressive approaches continue to be studied, successful immune tolerance to in-vivo gene therapy, to date, has been achieved using agents with multiple immune system targets. Immune responses to gene therapy create limitations on the number of gene therapy doses a patient may expect to receive, with the current understanding that the patient will likely be able to receive only one dose per lifetime. The efficacy of the single gene therapy dose is of paramount importance. A broader spectrum immune suppression may accommodate the present incomplete understanding of immune responses to gene therapy, and the consideration that patients will likely not be eligible for a second dose if the first dose is sub-therapeutic.
Abstract:

Astrocytes, the most numerous glial cell in the brain, are essential for normal neuronal function. In almost all brain diseases, astrocytes undergo a morphological and functional changes termed astrogliosis, yet the functional role of astrogliosis in disease remains unclear. Previous studies have identified two distinct reactive astrocytic phenotypes: A1 which is neurotoxic and A2 which is neuroprotective. While A1 reactive astrocytes can be induced by activating microglia with high doses of TLR4 agonist LPS, much less is known about induction of neuroprotective A2 astrocytes.

Spinocerebellar ataxia type 1 (SCA1) is a fatal neurodegenerative disease caused by CAG repeat expansions in the ATXN1 gene. SCA1 manifests clinically as the progressive loss of balance and coordination due to the dysfunction and degeneration of the cerebellum and its connected structures. Cerebellar astrogliosis is evident early in SCA1, prior to the onset of motor deficits and cell loss and throughout later stages of disease. Our previous work indicates that astrocytes are neuroprotective during early stages of SCA1 but become neurotoxic during late stages.

Here, we investigate whether astrocytes in SCA1 mice exhibit enhanced expression of A2 genes during early stages of disease, and conversely express genes characteristic of A1 genes during late stages. Furthermore, we test whether mild inflammation caused by low doses of LPS may approximate early stages of disease and induce expression of neuroprotective A2 genes. This may present the possibility of modulating microglial crosstalk to astrocytes in order to enhance neuroprotective astrocyte phenotypes in disease.
Poster Number: 27
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Subject Area: Gene Therapy Understanding
Keywords: Gene Therapy, Communication, Understanding, Education
Abstract Title: Enhancing Awareness and Understanding of Gene Therapy among Rare Disease Communities: A Research-Driven Roadmap

Abstract: Gene therapy is one of the most promising investigational treatments for rare diseases; however, the complexity of the technology poses significant communication and education challenges among patients, families and caregivers, advocacy groups and primary care physicians. This study examines gene therapy awareness levels, knowledge gaps and communication needs among audiences through an analysis of peer-reviewed literature, expert interviews, a patient survey, a one-year social and traditional media audit, and an assessment of current informational materials available via the internet. The results reveal a general awareness about and acceptance of gene therapy for rare and serious disease, but not an accurate understanding. For instance, a survey of rare disease patients are caregivers revealed the potential side effects of gene therapy (28%) and how gene therapy works (23%) were the most frequently cited areas of confusion. Expert interviewees agreed that the average physician does not fully understand gene therapy technology or treatment. The aggregate findings underscore the importance of fostering realistic expectations, encouraging a dialogue among and between audiences, using multiple media channels for engagement, and providing broad, basic education about genetics. We propose strategies that stakeholders across industry, academia, patient organizations and healthcare professionals could adopt, including establishing a common language or standard vocabulary for gene therapy. This vocabulary could then be adopted by various influencers and information sources serving the rare disease community, as well as the general public to help layer clear, consistent communication.
X-linked adrenoleukodystrophy (X-ALD) is the most common inherited peroxisomal disorder, characterized by progressive inflammatory demyelination in the brain that often precedes neurologic symptoms. Early treatment can arrest inflammatory progression and lead to better outcomes. In an attempt to describe the earliest findings in cerebral ALD, we identified 30 cases of ALD with initial reported normal scans from a database containing 222 ALD patients. Cases were then subdivided into three groups: those who had signs or cerebral disease on initial imaging, those who developed cerebral disease during the follow-up period, and those who did not. We reviewed these MRI studies to determine whether earliest signs of disease were recognized, the approximate brain volume affected by disease on initial positive scan and follow up scan, as well as if delay in diagnosis resulted in higher Loes score on follow up. We noted that the earliest signs of cALD were missed in 73% of cases despite the onset of disease in common areas of involvement. Follow up/initial scan volume ratios ranged from 1.02 to greater than 200. We also noted that multiple cases with delayed diagnosis had a higher follow up Loes score. Early findings are often subtle and easily missed on MRI and there is no standard for cALD screening. Our purpose is to evaluate current screening protocols and call to attention these early findings and so that treatment can begin before cerebral disease becomes severe.
Abstract:

Historically, mutations in KCNQ2-coded KV7.2 potassium channel have been associated with benign familial neonatal epilepsy. Recently, a more severe phenotype of early infantile epileptic encephalopathy characterized by intractable seizures, infantile spasms, and severe psychomotor impairment has been appreciated. This rare syndrome, which typically presents within the first week after birth, has only been identified in about 100 families worldwide. Seizures typically cease after several years of life, but patients experience significant developmental delays and poor neurologic outcomes. Our team was involved in the care of a patient who presented in the first week of life with intractable seizures. An epilepsy genetic panel returned positive for a pathogenic heterozygous mutation at KCNQ2 (variant p.Gly290Asp resulting in a G>A substitution) which has been described in the literature in association with a neonatal epileptic encephalopathy. The KCNQ2 channel was identified as a promising target for anti-epileptic medications, primarily because targeting this channel avoids adverse effects on KCNQ1 (a similar voltage-gated potassium channel present on cardiomyocytes). This phenomenon is explained by the presence of a tryptophan residue only present on KCNQ2 that forms a lipophilic binding-site for ezogabine, an anticonvulsant. A retrospective review of patients with KCNQ2 who were treated with ezogabine showed improvement in 3 of the 4 patients started on the medication before 6 months of age, and 2 of the 7 patients treated later, with no serious side effects observed. Ezogabine is not currently FDA approved and is currently only available for compassionate use. Targeted genetic evaluation enabled us to provide tailored therapy with oxcarbazepine and vigabatrin based on the current literature about KCNQ2 related epilepsy. However, in order to improve long-term neurodevelopmental outcomes for these patients, it is critical to diagnose accurately, initiate treatment early, and develop additional pharmacological interventions based on the disease specific pathophysiology.
Abstract:
To develop new avenues for therapeutic intervention, improved models of pediatric cancer are desperately needed. Here, we use induced pluripotent stem cells (iPSC) and genome engineering technologies to model sarcoma development with hopes of causing a paradigm shift in the way that pediatric cancers can be studied and treated. To this end we have implemented CRISPR/Cas9 to initiate genomic instability and loss of cell cycle control by knocking out genes and generating specific translocation events. We have established methods to uniformly differentiate iPSC to mesenchymal stem cells and osteoblast progenitors. Functional assays are ongoing to test the cells for a transformed phenotype and new results will be reported. We hypothesize that bottom-up models of pediatric sarcoma generated by inducing genetic changes and analyzing consequent phenotypes in a controlled and stepwise fashion will represent an improved platform for discovery of novel drug targets and high throughput drug testing.
Seizure emergencies (SE) are seizure clusters or prolonged seizures lasting at least 5 minutes. Rectal diazepam has been the mainstay of out-of-hospital SE management, but many older children and adults object to this therapy. Intranasal formulations potentially offer safe, effective, and more socially acceptable treatment options. Our group has developed a novel intranasal drug delivery system involving water-soluble benzodiazepine prodrugs that are admixed with converting enzymes at the time of administration. The studies presented herein investigated the pharmacokinetics of this novel system in rats. As a first iteration, avizafone, a water-soluble diazepam prodrug, was synthesized and combined with Aspergillus oryzae protease (AOP). A single dose of avizafone (equivalent to 1.0 mg/kg diazepam) and AOP was administered to rats. As a second iteration, avizafone was combined with human aminopeptidase B (hAPB). Single doses of avizafone (equivalent to 0.5, 1.0, and 1.5 mg/kg diazepam) and hAPB were given. An IV diazepam dose (1 mg/kg) was administered as the comparator. Diazepam concentrations were measured in brain and plasma samples at specified times ranging from 2 to 90 min post-dose. Both enzymes rapidly converted avizafone to diazepam. In the first iteration, mean (± SD) maximum diazepam concentration, 450 ng/mL (± 53.7 ng/mL), were attained at 5 min post-dose. In the second iteration, maximum plasma concentrations of 71.5 ± 9.3, 388 ± 31, and 355 ± 187 ng/mL and times to peak plasma concentration of 5, 8, and 5 min for each dose level, respectively, were attained. For the low, medium, and high dose levels respectively, bioavailabilities were 77.8 ± 6.0, 112 ± 10, and 114 ± 7 %. These studies demonstrate the feasibility of a novel delivery system for rescue therapy. The supersaturated DZP concentrations result in very rapid and complete absorption offering the potential of a faster onset of action.
Compromised genomic integrity, caused by functional deficits in DNA repair, can cause multiple human disorders. Inherited defects in nucleotide excision repair (NER), which removes bulky DNA lesions from the nuclear genome, can cause multiple diseases including xeroderma pigmentosum and Cockayne syndrome. Xeroderma pigmentosum (XP) is an autosomal recessive disease where patients have varying levels NER, depending on the complementation group and gene mutation. A key substrate of NER is UV-induced DNA damage. Thus, XP patients experience severe sunburn with minimal sun exposure and have a 10,000-fold increased incidence of skin cancer. XP patients may also develop cataracts, hearing loss, and neurodegeneration. Cockayne syndrome (CS) is a related disease where patients additionally have impaired neurological development but no skin cancer.

Timely diagnosis of XP and CS is prevented by the lack of a CLIA-certified lab able to do the functional assays required for diagnosis. Measurement of unscheduled DNA synthesis (UDS) after UV irradiation of patient cells is the gold standard for diagnosing NER-deficiency disorders. Historically, UDS was measured in patient dermal fibroblasts produced from a skin biopsy using radiolabeled nucleotides. More recently fluorescently-labelled nucleotides and microscopy are used to measure NER. However, both of these approaches are laborious, slow and require specialized equipment, impeding clinical utility of the assay.

To overcome this barrier, we developed an assay to measure UDS using peripheral blood mononuclear cells and flow cytometry to detect DNA synthesis following edU incorporation and Click-iT chemistry. The assay is rapid, inexpensive, and has already been used to diagnose new cases of XP and CS for NIH and pediatricians.
Gravitational insecurity (GrI) is a condition of life-long problems with balance and movement sensitivity, identified by occupational therapists but unknown to the larger health-provider community, that causes people to restrict their activities, fear heights and experience sometimes intense general anxiety. This study is an initial test of the hypothesis that adults with GrI have abnormalities in the brainstem vestibular velocity storage circuit. To date, standard clinical posturography of 6 subjects with such balance problems since childhood has shown a significant relative weakness in vestibular control of body sway, compared to normal-range sway control by optic flow or proprioception. Follow-up rotary chair testing shows a significant negative correlation between a 16 item GrI index of balance concerns and the time constant of the velocity storage circuit as measured by per-rotary nystagmus. Similar correlations with time constants were found for subjects reports of motion sickness and fear-of-falling on standard validated scales. If testing of additional subjects supports these results, and expected differences are found between this balance-challenged group vs. sex and age-matched comparison subjects, a basis will be provided for informing the medical community about this condition and developing therapeutic interventions.
Abstract: Hereditary tyrosinemia type 1 (HT1) is an autosomal recessive inborn error of metabolism that is characterized by the inability to breakdown tyrosine, causing an accumulation of toxic metabolites in the liver and resulting in severe oxidative damage. These patients develop fibrosis, cirrhosis, high rates of hepatocellular carcinoma and ultimately liver failure at a very young age if left untreated. Our group has previously demonstrated that ex vivo lentiviral (LV) gene transfers followed by autologous transplantation of corrected hepatocytes is curative in both mouse and pig models of HT1. The ex vivo approach, although effective, requires a partial hepatectomy in liver-based diseases. In vivo liver-directed gene therapy is an attractive non-surgical option for the treatment of genetic hepatic disease. In this study, we evaluated both effectiveness and safety profile of in vivo liver-directed LV gene therapy for the treatment of HT1 in pigs. Pigs dosed with LV-FAH (n=4) showed only a transient immune response to the lentiviral vector and transgene. They were able to become weight stable after only four cycles on the maintenance medication, NTBC. Blood data from 150 days post treatment showed normalized tyrosine and liver function enzyme levels. No increase in alpha-fetoprotein was seen, indicating a lack of hepatocellular carcinoma. Immunohistochemistry of livers from dosed pigs showed nearly complete repopulation of the liver with FAH+ hepatocytes at 6 months post treatment, with a reversal of fibrosis by 12 months. An analysis of the lentivirus integration profile showed a benign pattern, with a lack of preference for CpG islands or tumor coding genes. These safety and efficacy data indicate that lentiviral vector gene therapy should be considered for application to human patients.
We present an optimized processing pipeline for longitudinal DTI data analysis in spinocerebellar ataxia type 1 (SCA1) patients, a rare neurodegenerative disease. Tract-based spatial statistics (TBSS) analysis was used to investigate longitudinal changes in white matter (WM) integrity in patients. While no significant changes in WM integrity were observed using the standard TBSS analysis pipeline, TBSS with advanced spatial normalization with DTITK tool showed significant longitudinal alterations in WM integrity. This result suggests that the use of advanced spatial normalization must be considered for longitudinal group studies of DTI data, especially when small to moderate disease effects are expected.
Absence of dystrophin protein causes Duchenne muscular dystrophy, which is modeled by the dystrophin deficient mdx mouse. Compared to wildtype muscle, dystrophic mdx muscle is highly susceptible to eccentric contraction induced torque loss. Lack of dystrophin also influences the subsarcolemmal microtubule lattice. Wildtype muscle has a highly organized microtubule lattice of 90° intersections which becomes disorganized in mdx. Three additional mouse lines with disorganized microtubules are also susceptible to eccentric contraction induced torque loss, insinuating that microtubule lattice aberrations may contribute to the susceptibility of dystrophic muscle to eccentric contraction. Expression of mini- or micro-dystrophin transgenes in mdx skeletal muscle significantly rescues both eccentric contraction susceptibility and microtubule disorganization. Here we show that eccentric contractions can themselves also influence the microtubule lattice. While microtubule lattice organization of mdx muscle is significantly improved by any dystrophin transgene containing the N-terminus and cysteine rich domain, full lattice restoration requires either dystrophin spectrin like repeats R4-15 or R20-23. Mini-dystrophins containing R20-23 but lacking R4-15 can completely restore the microtubule lattice, however, micro-dystrophins lacking both regions only intermediately restore the lattice. Further, a chimeric dystrophin containing R4-15 but lacking R20-23 via substitution of homologous utrophin repeats, is also capable of restoring the microtubule lattice completely. Most interestingly, we find that both R4-15 and R20-23 appear to be required to fully protect the microtubule lattice from eccentric contraction. Lack of either dystrophin R4-15 or R20-23 results in partial transverse microtubule loss, and lack of both regions results in almost complete transverse microtubule loss while wildtype muscle suffers no microtubule loss after eccentric contraction.
Brain tumors have a grim prognosis following current treatment strategies of surgery, radiation therapy, and chemotherapy. Clinical efficacy of any chemotherapeutic agent depends on its potency and also its brain distribution for the treatment of the brain tumors as blood-brain barrier plays an important role in limiting the delivery of many chemotherapeutic agents [1]. Here, we evaluated brain distribution of a third generation topoisomerase-I inhibitors LMP400. Already existing topoisomerase-I inhibitors are camptothecin (cpt) derivatives suffer from lactone ring instability and reversible target site binding [2]. In vitro cytotoxic assays evaluated at our laboratory suggest that LMP400 is potent against various glioblastoma patient derived xenografts with IC50 values ~ 30-100 nM. We evaluated brain distribution of LMP400 in wild-type and Mdr1a/b/-/ Bcrp1/-/- (triple knockout) FVB mice (n=4) following intravenous, intraperitoneal and oral administration. Plasma and brain samples were collected at 7 time points post dosing and were analyzed using LC-MS/MS. The pharmacokinetic parameters were obtained by noncompartmental analysis (NCA) performed using Phoenix WinNonlin version 6.4. The total concentrations of LMP400 in brain were higher than in plasma, resulting in a brain-to-plasma AUC ratio (Kp) of 2.7 in wild-type mice. The brain distribution of LMP400 was modestly enhanced in triple knockout mice with brain-to-plasma AUC ratio (Kp) of 5.3. LMP400 plasma free fraction was found to be 1.32% and we are in the process of evaluating the free fraction in brain homogenate. The brain distribution characteristics of LMP400 make it an attractive cytotoxic agent for preclinical and clinical testing in brain tumors. [1] S. Agarwal, R. Sane, R. Oberoi, J.R. Ohlfest, W.F. Elmquist, Delivery of molecularly targeted therapy to malignant glioma, a disease of the whole brain, Expert reviews in molecular medicine, 13 (2011) e17. [2] J.L. Holleran, R.A. Parise, A.E. Yellow-Duke, M.J. Egorin, J.L. Eiseman, J.M. Covey, J.H. Beumer, Liquid chromatography-tandem mass spectrometric assay for the quantitation in human plasma of the novel indenoisoquinoline topoisomerase I inhibitors, NSC 743400 and NSC 725776, Journal of pharmaceutical and biomedical analysis, 52 (2010) 714-720.
Abstract: With the development of enzyme replacement therapy (ERT) and hematopoietic cell transplantation (HCT), children with mucopolysaccharidoses (MPS) types I, II and VI are now living into adulthood and long-term disease complications need to be considered. Growth failure and short stature are known features of these conditions; however, the long-term impact of ERT or HCT on height and weight are unknown. Height and weight were collected prospectively at annual visits in a 5-year longitudinal observational study of individuals with MPS types IH (n=25, 56% female), IA (n=8, 13% female), II (n=13) and VI (n=8, 25% female). Mean estimates for height SDS and BMI SDS were determined at baseline. The slope for change in height SDS and BMI SDS over 2-5 years was estimated. At baseline, children with MPS type IH (Age 9.4±3.5 years; Ht SDS -2.92±1.59) and VI (Age 14.1±5.5 years; Ht SDS -4.8±2.19) have severe short stature while children with MPS IA (Age 14.4±4.2 years; Ht SDS -1.53±1.22) and II (Age 9.8±2.7 years; Ht SDS -1.22±1.56) are less affected. The height SDS further decreased over time in children with MPS type II (slope -0.19 [95% CI:-0.3, -0.08; p=0.002]) and VI (slope -0.42 [95% CI:-0.59, -0.24; p b .001]). The mean BMI SDS are significantly elevated at baseline in all MPS (IH: 0.57±1.05, IA:0.62±1.35, II: 0.94±0.75, VI:0.57±1.19) and remains elevated over time. These prospective results revealed worsening linear growth over time in individuals with MPS type II and VI, and significantly elevated BMI SDS in all MPS groups. The elevated BMI in individuals with MPS is a risk factor for long-term cardiovascular disease. Additional long-term studies are needed to further understand body composition changes in individuals with MPS and determine potential mechanisms to improve BMI and modify cardiovascular risks.
Rett syndrome (RTT) is a neurogenetic syndrome that affects approximately 1 in 10,000 females. Preclinical have documented excessive cortisol responses to stress, but this has not been replicated in humans. This may be due to difficulties in identifying stress paradigms that can be ethically and practically implemented in this population. In the current study, we investigated cortisol responses to two standardized assessments designed to investigate musculoskeletal pain status and somatosensory function, respectively. 14 participants with clinical diagnoses of RTT participated (aged 4-38 years). Each research visit consisted of five segments: 5-minute baseline period; standardized range of motion exam; recovery period; modified standardized quantitative sensory test (mQST); and recovery period. Saliva samples were collected at three timepoints: prior to the first baseline (T1), following the sensory exam (T2), and at the end of the visit (T3). Linear mixed models were used to determine whether there were significant changes in cortisol across the three samples, and to evaluate differences in patterns based on age and parent-reported mood symptoms. On average, individuals with more parent-reported mood symptoms had marginally higher cortisol levels at T1, with no significant changes in concentration across the visit. Individuals with lower mood symptoms showed a significant increase in cortisol levels from T1 to T2, followed by a drop at T3. The results for age suggested that younger individuals showed significant increases from T1 to T2, and from T2 to T3, whereas older individuals showed no significant changes.

These results suggest that both the standardized range of motion exam and the mQST protocol produce significant changes in cortisol concentrations among individuals with RTT, although the specific patterns differ based on both age and mood symptoms. Overall, these findings suggest that these assessments may be feasible and useful in the assessment of stress reactivity among individuals with RTT.
ESETT is a randomized, double-blind trial comparing fosphenytoin (FOS), levetiracetam (LEV) and valproic acid (VPA) in patients with established status epilepticus (ESE). An ancillary study will characterize how plasma drug concentrations relate to the likelihood of seizure cessation in children and this study utilizes a sparse sampling approach: 1 sample collected within 20-50 min and the other within 60-120 min after the start of drug infusion. The objective of this work is to characterize the performance of this sparse sampling approach to predict partial area under the curve from 20-120 min (pAUC). Literature-based population pharmacokinetic (PK) models were used to simulate 2 types of rich concentration-time profiles, without error (true) and with error, for 500 pediatric patients (8 to 75 kg) for each drug (20 mg/kg FOS PHT-equiv, 40 mg/kg VPA, or 60 mg/kg LEV i.v. over 10 min). One timepoint and corresponding concentration with error was randomly selected from each sampling window (20-50 min and 60-120 min) for 100 randomly selected simulated patients. We then developed population PK models using the 200 concentrations. The percent prediction error (PPE) for pAUC prediction was calculated as: [(true-sparse)/true]*100. As an alternative approach, the concentration at the randomly sampled timepoint in the first window C1 was also compared with C60. R was used for simulations (mrgsolve), statistical analyses and graphing and NONMEM v 7.3 (Icon Ltd) for modeling. Despite using mg/kg dosing in children, ~3-fold variability in predicted exposure measures was found for all 3 drugs. For the sparse sampling approach, the PPE was within 30% for PHT and LEV and within 40% for VPA. Using C1 as an estimate of early exposure was inferior to the sparse sampling modeling approach and led to bias in prediction. We conclude that a sparse sampling approach can accurately predict metrics of early drug exposure and will allow for exposure-response modeling to enable further investigation of factors affecting drug response in children with ESE.
Abstract: The limiting factor in clinical application of gene therapy is no longer the identification of clinically useful gene targets, but the lack of highly specific delivery systems. Adeno-associated virus (AAV) is an appealing vector for human gene therapy because of its small size and low immunogenicity, but its natural tropisms are not typically clinically relevant and are not easily modified using traditional protein engineering techniques. We are working to create a system for rapid, covalent attachment of an antibody to a tropism-null viral vector using small ssDNA binding proteins and commercially available oligo-antibody conjugation technology. We are pursuing multiple methods of retargeting AAV by encoding various binding proteins in the capsid.
Abstract: Background. Newborn screening (NBS) for mucopolysaccharidosis type I (MPS I) allows initiation of early treatment for infants with known mutations. For those with unknown mutations, treatment strategies become complicated as early enzyme replacement therapy (ERT) may subtly alter clinical findings, preventing identification of infants with severe mutations requiring hematopoietic cell transplantation (HCT). Recognizing features of severe mutations by neuroimaging could be a useful tool for classifying severity.

Methods. MRI images from 12 infants < 6 months of age with known severe MPS I were reviewed for features known to be present in severe MPS I. These included: perivascular space enlargement, enlarged extra-axial spaces, white matter lesions, ventriculomegaly, bony abnormalities of skull and cervical spine, cervical cord stenosis, and odontoid capping. Additionally, dilated optic nerve sheaths and mastoid fluid were noted, when present.

Results. Infants (6 male) were studied at a median of 76 (21-185) days. Six infants had begun ERT a median of 13 (range 2 - 87) days prior to imaging. No infant had a normal scan. Bony abnormalities of the skull (12/12), the presence of mastoid fluid (12/12), and enlarged perivascular spaces (10/12) were the most common findings. Enlarged extra-axial spaces (8/12); cervical spinal canal stenosis (8/12); enlarged optic nerves (6/11); ventriculomegaly (7/12); and odontoid capping (7/12) occurred in >50% of infants. White matter lesions (0/12) and cortical atrophy 0/12) were not identified.

Conclusions. Infants <6 months of age with severe MPS I have neuro-imaging findings known to be present in older children with MPS I, most commonly bony abnormalities of the skull and enlarged perivascular spaces. Evaluation of a comparable group of attenuated MPS I infants will be crucial before employing these findings as part of the assessment of disease severity in unknown mutations.