NCL Research Newsletter October 2020

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1. Main Topic

1.1 Hope for CLN3 antisense therapy

Several therapeutic applications of **antisense oligonucleotides (ASOs)** have received market authorization by regulatory authorities in the United States and the European Union. Both the early and the late ASO drug pipelines are rapidly growing and rare genetic diseases are a major focus of ASO therapy (for recent review see: Bennett, 2019). In our last newsletter we cited a "N-of-1" CLN7 patient customized ASO trial which aims at correcting miss-splicing of the CLN7 mRNA in a single patient with a so far unique mutation in CLN7.

ASOs for therapeutic application include several that are designed to modulate splicing and restore protein function. Examples include the **exon skipping ASO drug** Eteplirsen for Duchenne Muscular Dystrophy, and the ASO drug Nusinersen for Spinal Muscular Atrophy which promotes exon exclusion. Many NCL forms are genetically quite heterogenous because of a wide spectrum of disease-causing mutations. CLN3 disease is an interesting exception because the vast majority of CLN3 patients (~80%) carry at least one mutant allele with a deletion of exons 7 and 8. Hence, a large subset of CLN3 patients could potentially benefit from a therapy that aims at restoring CLN3 protein function from the mutant $CLN3^{\Delta ex7/8}$ allele.

The deletion of two exons in the mutant $CLN3^{\Delta ex7/8}$ allele results in a spliced mRNA that encodes the wildtype CLN3 amino acids of exons 1-6. Splicing of exon 6 to exon 9 results in a change in the reading frame and a premature stop codon in exon 9. As a result, the mRNA of the mutant $CLN3^{\Delta ex7/8}$ allele only encodes the first 181 amino acid of the wildtype CLN3 protein and a short novel C-terminal amino acid tail. It lacks the C-terminal 257 amino acids of the wildtype CLN3 protein including lysosomal targeting sequences. Therefore, this truncated protein cannot properly traffic to the lysosome, and is degraded in the endoplasmic reticulum. In the hope to **partially correct CLN3 protein function** Centa and colleagues (Centa et al, 2020) developed ASOs that allow skipping of exon 5. These ASOs allow the production of an mRNA from the $CLN3^{\Delta ex7/8}$ allele that lacks exons 5, 7 and 8 correcting the reading frame, and give rise to a mutant $CLN3^{\Delta ex5/7/8}$ mRNA that encodes a big part of the C-terminal portion of the wildtype CLN3 protein. The authors present a hypothetical topology model of this truncated CLN3 protein. They also present several lines of evidence to support their working hypothesis that the $CLN3^{\Delta ex5/7/8}$ protein can at least partly compensate for the loss of wildtype CLN3 function. For example, they quantified and compared autophagosomes and autophagic flux in cells expressing WT, $CLN3^{\Delta ex7/8}$, or $CLN3^{\Delta ex5/7/8}$ constructs and found that only $CLN3^{\Delta ex7/8}$ altered autophagic parameters whereas $CLN3^{\Delta ex5/7/8}$ and WT CLN3 did not. They designed a series of 18-mer ASOs that base-pair in exon 5 with the aim to skip exon 5 and identified several ASOs that induced dose-

dependent exon 5 skipping in human heterozygous CLN3$^{\Delta ex7/8}$ fibroblasts and homozygous CLN3$^{\Delta ex7/8}$ cell lines. They also obtained evidence that CLN3$^{\Delta ex5/7/8}$ mRNA is more stable than CLN3$^{\Delta ex7/8}$ mRNA, and that ASO-mediated exon 5 skipping of CLN3$^{\Delta ex7/8}$ normalizes autophagic defects in homozygous CLN3$^{\Delta ex7/8}$ mutant cells. To provide further therapeutic support *in vivo*, they designed, screened and identified ASOs that skip exon 5 in CLN3$^{\Delta ex7/8}$ mouse cells and in adult CLN3$^{\Delta ex7/8}$ mutant mice after delivering ASOs directly to the central nervous system by intracerebroventricular (ICV) injection. To assess an effect of ASO induced exon 5 skipping on disease-associated outcome, postnatal day 1 or 2 mice were treated ICV with the most efficient exon 5 skipping ASO and compared to a separate group of animals that received a control ASO. Stable exon 5 skipping was observed to last for up to 14 months. The therapeutic ASO treatment lowered the accumulation of subunit C storage material, it lowered astroglia activation seen in untreated and control ASO treated mutant mice, and it significantly improved performance in two motor tasks. Finally, the authors introduced into the CLN3 mutant mouse background a **transgene encoding a familiar Alzheimer Disease (FAD)-linked human amyloid precursor protein (hAPP)**. This exacerbated disease and shortened life span down to 50% survival at 18.5 days. Treatment with the exon 5 skipping ASO significantly (186%) increased the 50% survival rate to 53 days. Altogether, this paper demonstrates the therapeutic potential of a **splice-switching ASO** targeting the most common CLN3$^{\Delta ex7/8}$ allele.

Open questions

This paper provides proof of principle for an ASO-based reading-frame correction exon-skipping therapy in CLN3 patients with the common CLN3$^{\Delta ex7/8}$ allele. The authors mention that work is ongoing to identify the best possible clinical candidate ASO. Open questions that remain are whether exon 5 skipping provides the only and best option to restore CLN3 function and whether therapeutic benefits can also be obtained if treatment starts after the onset of symptoms. Also, it would be desirable to continue and obtain more direct evidence for the ASO-based restoring of lysosomal membrane CLN3 protein expression and CLN3 function in the context of the neural network. We hope that the discovery of CLN3 disease-specific biomarkers will greatly facilitate such ASO as well as other proposed therapies for CLN3 disease.

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1.2 Biomarkers in demand to facilitate clinical validation of therapeutics in CLN3 disease

In our previous newsletter we mentioned that no clinically validated **NCL disease-specific biomarkers** have been identified to day. Therefore, clinical studies for testing new therapies in CLN3 disease will for the moment have to rely entirely on clinical outcome measures. An already excellent and still growing natural history of disease database with more and more disease-associated parameters being defined is a great asset. Nonetheless, the great bandwidth and patient-to-patient variability in onset, severity and progression of most of the disease-associated parameters remains a big challenge to define clinical end points for

trials in CLN3 disease. It weighs both on the number of patients that will have to be recruited in trials and the duration of trials.

At the 2020 LND-WORLD meeting in Orlando the NCL Foundation hosted a workshop for companies, government and academic institutions. The goal was to define common areas of interest to facilitate CLN3 clinical trial design and trial readiness. Four main areas were defined as of common interest: 1. Biomarker discovery and development, 2. Natural history of disease, 3. Clinical end points, and 4. Patient, family and caregiver perspectives. To discuss the first topic in more depth, we organized a **webex conference** that took place August 28 with over 40 participants. Several representatives from academia and government institutions were invited to break the ice and touched on **CLN3 biomarker discovery**. David Sleat (Rutgers University) reported on human and mouse brain and **CSF proteomic analysis** (see previous newsletter for more details). Jill Weimer (Sanford Health) explained their approach to not look for the needle in the haystack but rather at the whole haystack i.e. a multiparameter and multidimensional approach to identify a **biomarker fingerprint**. From Nationwide Children's Hospital Nicolas Abreu presented their biospecimen sampling strategy and Julieth Andrea Sierra Delgado the utility of **iPSC-derived astrocytes** for defining cellular phenotypes. Daniel Forbes Porter (NIH) presented lessons learned from biomarker efforts in Niemann Pick type C disease, and An Dang Do (NIH) explained their ongoing IRB-approved longitudinal **Natural History Study** of CLN3 patients which includes a unique CSF sample collection and extensive clinical evaluation. Henrik Zetterberg (University of Göteborg), a leading expert in the biomarker field of adult onset neurodegeneration (ND) furthermore discussed opportunities to align and profit from ongoing biomarker activities in the adult onset ND field. There was general agreement that the limited availability of samples and patient cohorts calls for **collaborative efforts** to maximize chances for biomarker identification. It also underlined the importance and the need for starting prospective sample collections in patient centers and accelerating IRB approvals.

The **analysis of CSF** rather than serum, plasma or urine is likely to provide a first best shot to identify CLN3 disease biomarkers, although none of the other sources can a priori be excluded. Also, it remains to be seen how valuable the cross-species CSF and blood translational biomarker of neurodegeneration, **neurofilament L chain (NF-L)**, will be in preclinical models of CLN3 disease, and more importantly in CLN3 patients. In CLN2 disease, NFL was shown to be a treatment-responsive biomarker (Ru et al 2019). Looking forward, the **biomarker workshop** facilitated and sparked new collaborative efforts towards both sample collection and biomarker identification in CLN3 disease. We hope that this will be fruitful and contribute to the success of CLN3 clinical trials.

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1.3 Interview with Prof. Pierluigi Nicotera

Prof. Dr. Dr. Pierluigi Nicotera is a renowned scientist and internationally leading expert in the field of neuronal cell death. Since April 2009 he is Scientific Director of the German Center for Neurodegenerative Diseases (DZNE). His research focuses on the molecular mechanisms leading to neuronal death after chronic and acute insults. The loss of neuronal synaptic connections and apoptosis plays a central role in neuro-degenerative diseases.

Prof Dr. Dr. Pierluigi Nicotera (DZNE) © DZNE

1. Prof. Jutta Gärtner (DZNE, Göttingen) recently received the Hamburger Science Award 2019 for her groundbreaking work in the field of rare congenital neurometabolic diseases in childhood and adolescence. Where do you see more mutual benefit of research on neurodegenerative diseases in childhood, adolescence, and adulthood? What and where can they learn from each other, also taking into account that lysosomal defects seem more and more a common theme?

Jutta Gärtner is an internationally recognized leader in the field of neurodegenerative diseases. Within the DZNE, she collaborates with other scientists engaged in understanding the causes and potential treatment of neurodegenerative diseases. The biological mechanisms underlying neurodegenerative diseases are different for each disease but also share some common pathways, including the important lysosomal “waste disposal” functions within cells. In addition to the pathways involved in protein processing and storage, other mechanisms such as inflammation and mitochondrial dysfunction can contribute to neurodegenerative diseases in all age groups. Therefore, it is important to promote interactions among scientists and clinicians whose work is in the traditionally separated areas of pediatric and adult/geriatric brain health.

2. Artificial Intelligence and Big Data is a big theme in medicine and drug development – where do you see the major benefit for neurodegenerative diseases in general, and more specifically, for areas like diagnostics, biomarkers, and discovering new therapeutics?

The future of medicine relies upon studies based on highly complex human data (such as cognitive assessments and imaging) and analysis of biospecimens (such as genomics and metabolic or inflammatory biomarkers). Dealing with the huge amount of data generated in modern human studies can only be approached by new learning strategies. The DZNE for example has pioneered studies using swarm learning and new computing technologies in collaboration with HPE (Hewlett Packard Enterprises). Thanks to new developments in machine learning and memory-based computing, we can manage such complex data to identify new markers that will help to define specific disease subtypes, and can yield better outcome measures to be used in clinical trials. This will be possible using edge computing, where patient data are not directly shared on the cloud but where computer modeling learns from data stored at individual site, thus offering the advantages of markedly faster computing times and enhanced data security.

3. Future outlook: there is a surge in ASO and gene therapy trials for rare monogenetic (including neurodegenerative) diseases. Even gene editing, although technically still very challenging for CNS application, is being talked about. Where do you see the chances as well as the major challenges still to be overcome for neurodegenerative diseases?

ASO and gene editing offer exciting opportunities for disease prevention and treatment, particularly in genetic diseases, and in children who are in a presymptomatic or very early stage of disease manifestation. However, these technologies are challenging to administer and extremely costly. In particular, caution should be exercised for the potential side effects of new gene-editing therapies (for example, effects induced by inadvertent editing of DNA sequences or cells types different from the intended target). Therefore, in parallel to pursuing these opportunities we should continue to search for other pharmacological or biological interventions.

4. Do you see research areas where the DZNE and the NCL Foundation could mutually benefit and work together more closely?

We already collaborate successfully in research areas that focus on disease-overarching mechanisms and we value this collaboration very much. As noted above, we should both continue to strive to synergize the joint efforts of researchers in childhood and adult neurodegenerative diseases. One valuable approach is to bring internationally leading researchers together to explore overlapping areas of interest, as was done at the joint NCL Foundation-DZNE Symposium several years ago. This type of live group interaction is currently more difficult to achieve in these pandemic times, however it is particularly important now not to lose sight of the impact that neurodegenerative disease research can have on society.

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2. Scientific and Medical Meetings

December 6-7, the NCL Foundation will host the virtual **6th JNCL Young Investigator Symposium**. A live meeting was originally planned in conjunction with the 2020 intl. NCL congress in October in St. Louis, USA. This 17th Intl. Congress on the Neuronal Ceroid Lipofuscinoses (Batten disease) has been postponed to the fall of 2021. We will try and host a satellite conference for the CLN3 young investigators in conjunction with this meeting in 2021. Under the present COVID-19 circumstances changes cannot be excluded. Therefore, we advice to follow the updated info on the NCL2021 website.

The Central and Eastern European Society of Technology Assessment in Health Care (CEESTAHC) was founded in Poland in 2003. CEESTAHC organizes trainings and conferences on topics related to evidence-based medicine (EBM), health technology assessment (HTA) with the main aim to develop and progress standards and methods of assessment of drug and non-drug health technologies in Central and Eastern Europe. The **15th Intl. Evidence-Based Health Care Symposium** titled "From Evidence to Action" was held online October 5-7, 2020. Focus was on non-cancer rare diseases and the use of multi criteria decision analysis (MCDA) in the reimbursement decision process with the aim to improve real world situations.

Relevant to this topic also, is a recent publication by the European Reference Network for hereditary metabolic diseases MetabERN. A survey was performed asking health care providers from 18 European countries whether products are available on the market, reimbursed and accessible for prescription, and yes/no delivered to centers.

Recently, the 10th annual Sanford virtual rare disease symposium took place (October 16). It covered topics including the role of autophagy in beta propeller protein-associated neurodegeneration, patient-driven therapies for rare diseases, gene therapies for inherited nervous system disorders, the involvement of astrocytes in rare neurological diseases, and the importance of glycosylation of a recombinant enzyme to effectively deliver ERT for Pompe disease. Also, a number of posters were presented. Relevant to NCL, an update on the CLN3 porcine model (Weimer lab.), overlapping protein interactome for CLN3, CLN6 and CLN8 (Weimer lab.), and Sigma-1 receptor agonists as potential therapies for CLN6 Batten disease (Kovacs lab). For details see here.

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3. Grants and Awards

Recently the NCL Foundation started funding a new NCL research project in Prof. Dr. Dr. Andreas Guse's lab (University Clinic Hamburg-Eppendorf, Germany). The foundation supports a fellowship for Sukanya Arcot Kannabiran, M.Sc.

Dr. Frank Stehr (CEO NCL Foundation) and Sukanya Arcot Kannabiran, M.Sc. (UKE), © A. Kirchhof / UKE

The endo-lysosomal system is a genetic hotspot in both adult onset and childhood onset neurodegenerative diseases (ND). Over the last decade many links have emerged between pediatric lysosomal storage diseases (LSD) and adult-onset ND. This is not surprising because the endo-lysosomal pathway is at the crossroad of vital cellular processes. These include endocytosis, exocytosis, cargo degradation, autophagy, nutrient sensing and regulating adaptive cellular processes, the recycling of metabolites and their exchange with other organelles which often involves organellar contact sites.

Calcium is known to play an incredibly important role in the overall regulation and dynamics of the endo-lysosomal system. For example, in processes such as vesicle trafficking, vesicle fusion, and vesicle exocytosis. In many cases the lysosome itself has emerged as a Ca^{2+} signaling center and disrupted lysosomal Ca^{2+} signaling is believed to play a key role in several LSDs such as Niemann Pick type C1, Mucolipidosis type IV, CLN3 and CLN12 disease, as well as adult-onset NDs. A more in-depth understanding of the role of endo-lysosomal Ca and the channels regulating its Ca homeostasis is of central importance. Also, to advance novel therapeutic concepts such as those that target some of these channels. High resolution Ca imaging techniques and tools are needed to advance our understanding of the role and dynamics of lysosomal Ca microdomains in processes underlying diseases including CLN3 Batten disease. The lab of Prof. Andreas Guse and his Calcium Signaling Group at the UKE in Hamburg dedicate their research to resolving questions related to Ca signaling and the role of Ca microdomains. Dr. Kannabiran will apply state-of-the art tools and technologies to try and resolve some of the questions related to Ca microdomain signaling and proposed therapeutic targets in CLN3 disease e.a. NDs.

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4. NCL News & Recent Publications

CLN1

Abeona Therapeutics Inc. and Taysha Gene Therapies announced August 17 that they have entered into license and inventory purchase agreements for ABO-222, an AAV gene therapy for CLN1 disease.

Balouch et al. reported that human CLN1 fibroblasts harboring Met1Ile and Tyr247His compound heterozygous mutations have a heightened susceptibility to exogenous reactive oxygen species (ROS)-induced cell death.

Nelvagal et al. reported early and progressive spinal cord pathology in CLN1 mice. Proteomic changes in the spinal cord and cortex at 3 months revealed many similarly affected processes. However, the site-specific nature of inflammation in CLN1 disease may help explain the limited success of previous brain-directed therapies.

Gorenberg et al. used quantitative mass spectrometry proteomics of PPT1 knockout mouse brain to identify substrates of the depalmitoylating enzyme PPT1. They found many sites of depalmitoylation in extracellular domains of transmembrane proteins facing the synaptic cleft, as well as several other NCL proteins.

CLN2

Whiting et al. evaluated the efficacy of periodic intravitreal injections of recombinant human TPP1 in inhibiting retinal degeneration and preserving retinal function in the CLN2 canine model. Treatment began at 12 weeks of age. Treated eyes in contrast to vehicle treated eyes retained normal morphology at end stage neurologic disease (43-46 weeks) demonstrating that periodic intravitreal ERT may preserve retinal structure and function.

REGENXBIO reported expansion of their pipeline to include the AAV9-TPP1 vector RGX-381 to deliver the TPP1 gene directly to the **retina** for the treatment of ocular manifestations of CLN2 disease. This complements their existing RGX-181 gene therapy program for the treatment of CLN2 disease in the central nervous system. The company expects to submit an IND for the intracisternal delivery of RGX-181 by the end of 2020, and plans to initiate enrollment in a Phase I/II trial in the first half of 2021.

CLN3

BBDF & Theranexus: In August 2020, the FDA awarded Orphan Drug and Rare Pediatric Disease designation to the Beyond Batten Disease Foundation for BBDF-101. This designation provides accelerated review for approval. It does not impact on the clinical trial but it is beneficial once the therapy receives New Drug Approval (NDA).

Centa et al. showed therapeutic efficacy of an antisense oligonucleotide that promotes exon 5 skipping in the CLN3$^{\Delta ex7/8}$ mouse model of CLN3 Batten disease. For details see section 1.1.

Masten et al. propose a novel CLN3 disease Staging System to classify individuals into specific strata based on age and disease severity. It has potential applications in clinical trials for cohort stratification.

Kuper et al. quantified vacuolization in lymphocyte subsets of CLN3 disease patients showing that this can serve as a measure of disease severity. Using flow cytometry and detection of intracellular LAMP-1 as a proxi the phenomenon was most pronounced in T-cells of patients with classical or protracted CLN3 disease while in “retina-only” CLN3 disease it was most pronounced in B-cells.

Seifert et al. described that CLN3 interaction with the voltage-gated potassium channel (Kv) interacting protein KChIP3 suppresses the typical KChIP3-mediated modulation of Kv4.2, and these effects were weaker for the CLN3 disease mutant proteins R334C and a C-terminal deletion mutant. In brief, CLN3 might be involved in proper Kv4.2/KChIP3 somatodendritic A-type channel formation, trafficking and function.

Zhong et al. reported that loss of CLN3 leads to metabolic impairment and autophagy induction in retina pigment epithelium (RPE) suggesting roles of CLN3 in maintaining healthy RPE and normal vision.

Dulz et al. described a newly established Hamburg CLN3 ophthalmic rating scale to assess ocular involvement in juvenile CLN3 disease. The authors show that ophthalmic manifestations correlate closely with the severity of neurological symptoms and age of the patient. The scale may serve as an objective marker of ocular disease severity and progression. It may also be useful in the evaluation of novel therapeutic strategies for CLN3 disease.

Wang et al. describe a detailed ophthalmoscopic, spectral and morphological analysis of focal autofluorescent and subunit C positive lesions in all layers of the $Cln3^{\Delta ex7/8}$ mouse retina. This occurs in mice at 8 months and older, predominantly in the retinal outer plexiform and inner nuclear layers.

Kleine Holthaus et al. demonstrated that $Cln3^{\Delta ex7/8}$ mice have a decline in inner retinal function resulting from the death of rod bipolar cells and interneurons. This phenotype is treated by AAV-mediated expression of CLN3 in cells of the inner retina which leads to a significant survival of bipolar cells and preserves retinal function. Targeting photoreceptors gave no therapeutic benefit. The authors propose that at least in this CLN3 mouse model, the disease is primarily a disease of the inner retina.

Bartsch et al. published a short review of preclinical studies on therapeutic strategies for the treatment of retinal dystrophy in animal models of different NCL forms including CLN3.

Huber published a review on findings in Dictyostelium and mammalian models of NCL proposing an integrated view of understanding the molecular network of NCL proteins.

Mizobuchi et al. described a novel *CLN3* missense variant (Ser161Leu) in a 26-year old Japanese patient that is associated with teenage-onset isolated retinal dystrophy but no neurological signs during the 13-year follow-up period.

Kuper et al published a medical chart review of 38 children with either CLN3 or early-onset Stargardt disease (STGD1). These children had been referred to a specialized ophthalmological center because of rapid vision loss. The authors concluded as early clinical features differentiating between the two diseases a more rapid loss of visual acuity, severe color vision abnormalities, and abnormal dark-adapted ERG responses in CLN3 disease.

Maalouf et al presented experimental evidence for therapeutic effects of Flupirtine in CLN3-deficient cells and $CLN3^{\Delta ex7/8}$ mice. Presented are beneficial impacts on motor behavior, learning & memory, anti-apoptotic gene expression, and astrogliosis. Also described are some effects of a new allyl carbamate derivative of flupirtine.

Kinarivala et al expanded the limited repertoire of *in vitro CLN3 cell* models suitable for drug screening by generating a novel $CLN3^{\Delta ex7/8;Val330Phe}$ iPSC-derived neuron and brain microvascular endothelial cell model. Using the former model, they identified mTOR-independent autophagy modulators that cleared subunit c accumulation and rescued mitochondrial dysfunction. In their CLN3 iPSC-derived blood brain barrier model they show an impaired barrier function.

Qureshi et al review and discuss the evidence for a shared retromer-dependent endosomal trafficking pathway between some of the NCLs including CLN3, PD and AD and that link this pathway and others causing endo-lysosomal dysfunction.

Langin et al. describe a novel $Cln3^{Q352X}$ mouse model containing a non-sense mutation at amino acid position 352. Similar to previous CLN3 mouse models, these mice show CNS pathological deficits including accumulation of lysosomal storage material and glial activation, and limited perturbation in behavioral measures. This mouse line provides a tool for testing nonsense suppression and read through therapies.

CLN4

Imler et al. expressed autosomal dominant mutant human CLN4 (which encodes the synaptic vesicle protein CSPα) in Drosophila neurons. This induced excessive oligomerization of hCSPα, dose-dependent premature lethality, abnormal pre-lysosomal accumulation of hCSPα, and abnormal membrane structures in axons and neuronal somata. Enhancing endogenous wildtype dCSPα levels worsened while reducing its levels attenuated most phenotypes. Reducing Hsc70 gene dosage also attenuated CLN4 phenotypes. The authors propose that CLN4 alleles behave like dominant hypermorphic gain of function mutations.

CLN5

Luebben et al. deposited the crystal structure of the human CLN5 protein in the protein databank.

Luo et al. reported a novel CLN5 homozygous missense mutation (Arg145Pro) in a 5-year-old female patient that presented with paroxysmal epilepsy, progressive regression in walking, vision, intelligence and speaking. Evidence is provided of impaired cellular trafficking of this CLN5 mutant protein to the lysosome, indicating that this mutation is pathogenic.

CLN6

On Sept 24, it was announced that **Amicus Therapeutics** received European Medicines Agency (EMA) PRIME Designation for CLN6 Batten Disease Gene Therapy based on data from the ongoing Phase 1/2 clinical trial evaluating a single dose of AT-GTX-501 for the treatment of children with CLN6 Batten disease (see ClinicalTrials.gov; NCT02725580). The EMA PRIME initiative provides enhanced support and increased interaction to developers of promising medicines. The goal is to optimize development plans and speeding regulatory evaluations to help patients benefit as early as possible from innovative new therapies that have demonstrated the potential to significantly address an unmet medical need.

Bajaj et al. identified CLN6 as an obligate component of a CLN6-CLN8 complex (EGRESS: ER-to-Golgi relaying of enzymes of the lysosomal system). This complex recruits lysosomal enzymes at the ER to promote their Golgi transfer. The authors show that CLN6 deficiency results in inefficient ER export of lysosomal enzymes and diminished levels of the enzymes in the lysosome.

CLN7

Connolly et al. used the Drosophila larval neuromuscular junction (NMJ) to study the role of CLN7 in neural development. They demonstrate, that CLN7 is required for normal growth of NMJ synapses. CLN7 expression is restricted to the postsynaptic cell and the protein localizes to vesicles adjacent to the post-synaptic membrane suggesting that CLN7 might play a role in regulating trans-synaptic communication.

Crain et al. described a 5-year-old male patient who presented for MRI following a delay in achieving developmental milestones and epilepsy first diagnosed at age 3. A thinned corpus callosum and generalized low parenchymal volume with periventricular gliosis was observed while Magnetic Resonance Spectroscopy revealed glutamate/glutamine accumulation and diminished N-acetylaspartate.

CLN8

Johnson et al. tested in a mouse model of CLN8 (CLN8^{mnd}) the safety and efficacy of neonatal stage intracerebroventricular-delivery of a self-complementary AAV9 gene therapy vector driving expression of human CLN8. Treatment was safe and well-tolerated. Transgene expression was seen throughout the brain and spinal cord and lasted up to 24 months. It reduced histopathological and behavioral hallmarks of the disease and completely restored lifespan from 10 to beyond 24 months of age.

CLN11

Huang et al. performed proteomic analysis of brain from wild type and Progranulin knockout mice at 3- and 19-months of age. The findings indicate that lysosomal dysregulation is exacerbated with age in the knockout mouse brain leading to neuroinflammation, synaptic loss, and decreased markers of oligodendrocytes, myelin, and neurons. GPNMB and galectin-3 were upregulated by microglia and found to be elevated also in brain samples from patients with the GRN form of frontotemporal dementia (FTD-GRN). GPNMB levels were also significantly increased in the cerebrospinal fluid of FTD-GRN patients suggesting GPNMB is perhaps a biomarker specific to FTD-GRN.

Zhang et al. show that progranulin deficiency promotes microglial transition from a homeostatic to disease-specific state and a selective loss of excitatory neurons at disease end-stage. Neurons show nuclear and cytoplasmic TDP-43 granules and nuclear pore defects. Conditioned media from progranulin-deficient microglia promotes TDP-43 granule formation, nuclear pore defects and cell death in neurons. Deleting complement factors C1qa and C3 mitigates microglial toxicity and TDP-43 proteinopathy suggesting that endolysosomal dysfunction and chronic microglial toxicity contribute to TDP-43 proteinopathy and neurodegeneration.

Werner et al, **Zhou et al and Feng et al** all reported on findings related to the CLN11 (GRN) gene encoding the protein progranulin (PGRN). Homoyzgous deficiency of CLN11 is a very rare cause of NCL. Heterozygous mutations on the contrary are a common cause of frontotemporal dementia (FTD) and neuronal loss in adults. All three independent studies reported that complete removal of the lysosomal transmembrane protein TMEM106B exacerbates results in FTD-related defects and pathology including motor deficits, neurodegeneration, gliosis, lysosomal abnormalities, and TDP-43 pathology in mice lacking GRN. TMEM106B is a risk factor for FTD and other results had suggested that an increase, rather than a decrease in its expression causes the elevated risk **(Nicholson et al, (2013), Gallagher et al (2017), Klein et al, (2017). Clayton and Isaacs (2020)** discuss the findings and controversies in more detail, as well as their implications for proposed therapeutic routes for FTD to modulate TMEM106B levels.

Amado et al and **Hinderer et al** both present data on AAV-mediated progranulin delivery in mice, and the latter paper also in non-human primates. Amado et al. used AAV9 to deliver GRN to the lateral ventricles and observed hippocampal toxicity in neurons and glia preceded by T cell infiltration. The latter was also observed when delivering an ependymal targeting AAV. On the contrary, Hinderer et al. report no such toxicity related events, despite achieving high CSF progranulin levels, neither after delivery of AAV to the lateral ventricles in mice, nor after delivery to the cisterna magma in non-human primates. It seems rather unlikely that PGRN protein overproduction could explain these discrepancies but perhaps rather the use of different AAV types and/or technical aspects.

Tayebi et al present a working hypothesis that alterations in the cathepsin D (CLN10), prosaposin, Progranulin (CLN11) network and associated receptors modifies lysosomal function in Parkinson's Disease (PD) thereby impacting α-synuclein degradation and the activity of GCase encoded by the GBA1 gene. Both heterozygous and homozygous mutations in GBA1 are an important risk factor for PD and Dementia with Lewy Bodies.

Telpoukhovskaia et al present a potential therapeutic strategy for normalizing aberrant microglia function in GRN-deficiency. To this end the authors ran a compound screen coupled with high throughput sequencing to assess key transcriptional changes in inflammatory and lysosomal pathways, and in the end rescuing cathepsin activity. They identified two phenotypic modulators of progranulin deficiency that also rescued cell cycle abnormalities in progranulin-deficient cells.

CLN12

Van Veen et al. established ATP13A2 (CLN12) as a lysosomal polyamine exporter. Polyamines support diverse cell functions, are found in high concentrations in the mammalian brain, but at too high concentrations become toxic. The authors show that ATP13A2 promotes the cellular uptake of polyamines by endocytosis and transports them into the cytosol. This highlights a role for endolysosomes in their uptake. Polyamine-induced cell toxicity is exacerbated by ATP13A2 loss and proposed as a mechanism for lysosome-dependent cell death in neurodegeneration.