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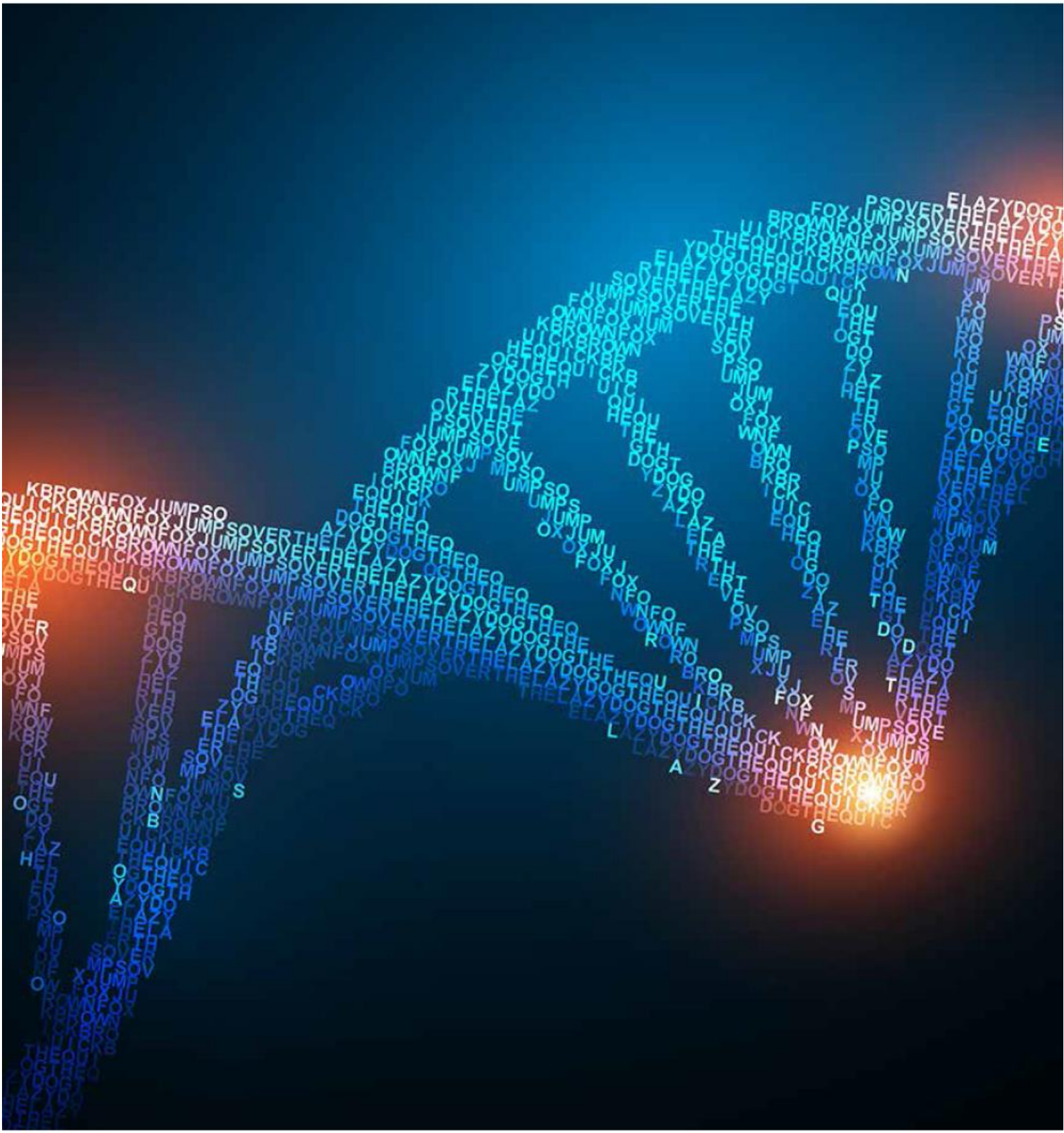

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ABSTRACT BOOK

The High-Throughput Screening and the NMN as Models of Potential Therapies for Mitochondrial Diseases

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Mitochondrial diseases are a large family of genetic disorders characterized by defects in multiple biochemical pathways and cellular processes in mitochondria. It is associated with a decrease in energy production and other essential functions not related to ATP synthesis, making it challenging to identify therapeutic procedures. We have afforded two therapeutics strategies with *Saccharomyces cerevisiae* as model, one specific for coenzyme Q deficiency and a second one for general mitochondrial defects.
The first strategy consists of a High-Throughput Screening (HTS) to detect small soluble molecules bypassing the defect in the synthesis of CoQ_6 in yeast. This method requires the stabilization of the CoQ biosynthesis complex (Q-synthome) by *COQ8* gene overexpression and monitoring the growth in a non-fermentable carbon source such as glycerol. The application of this pilot approach allowed us to explore a library of 1200 natural microbial extracts, finding nine positive ones and identify five molecules with therapeutic potential in coenzyme Q_{10} human deficiency.

The second strategy analyzes the potential benefits of modifying NAD^+ levels by supplementation with its precursor, the nicotinamide mononucleotide (NMN). NAD^+ has described as a regulator of mitochondrial metabolism and has emerged as a new strategy of therapy for mitochondrial diseases. We have studied the effect of NMN on mitochondrial metabolism and the possible pathways involved. Our results showed that NMN supplementation induces mitochondrial responses that depend on mitochondrial sirtuins activation Hst4. This activation drives changes of proteins associated with mtDNA-nucleoids; increasing the mitochondrial activity by the expression of mtDNA-encoded proteins.

Mass Spectrometry Imaging (MSI) Identifies Changes in The Region-Specific Distribution of Cerebral Lipids in A Mouse Model of Niemann-Pick-disease Type C1 (NPC1)

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NPC1 is a rare autosomal-recessive lipid transport and storage disorder in which the brain becomes affected by a selective, progressive dysmyelination and distinct neurodegeneration, but detailed information on affected functional regions is still lacking. We used frozen brains of *NPC1−/−* and control wild type male and female mice (n=10; 60-70 days old) as approved by governmental authorities (study-no. 7221.3-1-01-011/16). Frontal brain sections (10 µm) at 5 different levels were analyzed by Matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI LTQ-Orbitrap XL mass spectrometer at 100 µm lateral resolution) followed by cytoarchitectonic evaluation. SCiLS Lab MVS, version 2018, was used to identify discriminative peaks and generate MS images. Lipids were assigned from LIPID MAPS (http://www.lipidmaps.org) based on the high accuracy mass measurement. From the 103 lipids which were identified in positive and negative ion mode analysis, 57 appeared altered in *NPC1−/−* brains. GM2 (d18:1/18:0) and GM3 (d36:1) levels were significantly increased in multiple regions in *NPC1−/−* such as cerebral cortex but with highest levels in distinct subcortical regions e.g. amygdala, hippocampus (CA3) and olfactory bulb. Also significant region-specific accumulation of ganglioside GM1(d38:1) and phosphoinositol (PI 36:4) occurred with highest levels of GM1 confined to retrosplenial cortex, thalamus, cerebellar grey matter and for PI (36:4) in addition, in olfactory bulb, piriform cortex and corpus callosum but not in cerebellum. In contrast to the gangliosides, sulfatides (ST), such as ST (d18:1/24:1) levels were significantly reduced in several distinct forebrain regions except in cerebellar white matter in *NPC1−/−* brains. This indicates a wider range of cerebral lipid changes in brains of *NPC1−/−* mice which allows a functionally analysis and interpretation with specified behavioral tests and provides foundation for studying the target and off-target effects of therapeutic interventions in animal models.

N-Acetyl-L-Leucine for Niemann-Pick Disease, Type C, GM2-Gangliosidosis and Ataxia Telangiectasia: Three Multinational, Multicenter, Open-Label, Rater-Blinded Phase II Clinical Trials

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Introduction: N-Acetyl-L-Leucine (IB1001/ALL) clinical trials are three, phase II studies which investigate ALL for three rare, autosomal-recessive, neurodegenerative disorders: Niemann-Pick type C (NPC), GM2-Gangliosidosis (GM2) and Ataxia-telangiectasia (A-T). The mutual aspects of their clinical presentation, and mechanism of action of ALL, enabled a single master protocol to be developed, implementing both an innovative trial design and novel primary endpoint, better suited to these small, inhomogeneous patient populations.

Methods: The IB1001 studies investigate the symptomatic and disease-modifying effects of ALL. Screening of patients ≥6 years (Europe) AND ≥18 years (USA) occurs at 12 centers across Germany, Spain, Slovakia, UK, and USA. Patients who have completed the Parent Study (Fig.1 and 2) may be included into an Extension Phase (Fig. 3) The dosage varies from 2 to 4 g/day, based on patients' age/weight. A novel primary endpoint, the Clinical Impression of Change in Severity (CI-CS), was developed, based on two independent, blinded raters comparison of videos of the patient's change in performance from baseline to the end of treatment, and the end of treatment to the end of the washout on either the 8 Meter Walk Test (8MWT) or the 9 Hole Peg Test, Dominant Hand (9HPT-D).

Results: Recruitment is ongoing for all three studies. Approximately 39 patients per study will be screened. As of 15 January 2020, 29 NPC, 13 GM2, and 1 A-T patients have been enrolled.

Conclusions: Due to the clinical heterogeneity of these orphan populations, a novel primary endpoint is implemented to better demonstrate the clinically meaningful effect of ALL treatment.

Acetyl-Leucine Slows Disease Progression in Lysosomal Storage Disorders

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Introduction: Acetyl-DL-leucine (ADLL) is a derivative of the branched amino acid leucine. In observational clinical studies ADLL improved symptoms of ataxia in patients with the lysosomal storage disorder (LSD) Niemann-Pick disease type C (NPC).[1, 2] We investigated ADLL and its enantiomers acetyl-D-leucine (ADL) and acetyl-L-leucine (ALL) in *Npc1*$^{-/-}$ mice.

Methods: Affected mice (Npc1-/-, Hexb-/-) and controls (Npc1+/+, Hexb+/+) were included. Behavioral tests were performed (gait analysis, motor function assessment, incl. strength and coordination). Biochemical analyses (Western blot, ADP/ATP and NAD/NADH, sphingoid base, glycosphingolipid, cholesterol) were performed. Moreover, lysotracker green and propidium iodide staining, filipin staining as well as immunohistochemistry were conducted. Clinical observational studies of 13 adult NPC (12 on miglustat) and 3 GM2-gangliosidoses patients treated with ADLL were included.

Results: ADLL, ADL and ALL in symptomatic *Npc1*$^{-/-}$ mice all improved ataxia. When ADLL and ALL were administered pre-symptomatically to *Npc1*$^{-/-}$ mice, they both delayed disease progression and resulted in a modest extension to life span, whereas ADL did not. These data are consistent with ALL being the active neuroprotective enantiomer. Altered energy metabolism was implicated as a potential mechanism of action of the active L enantiomer in *Npc1*$^{-/-}$ mice. When miglustat and ADLL were used in combination significant synergy resulted. Disease progression rates were slowed after 12 months of treatment. A neuroprotective effect of ADLL was also observed in a mouse model, and clinical benefit observed in GM2 gangliosidosis` patients in observational clinical studies.

Conclusions: Taken together, we have identified an unanticipated neuroprotective effect of ALL, supporting its further evaluation in clinical trials in LSD.

Clinical Whole-Genome Sequencing, Co-Occurring Rare Diseases and Pharmacogenetic Profiling

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Background: In the current genomics era, interpretation of high-throughput sequencing data constitutes the main bottleneck on the path to accurate diagnosis of Mendelian disorders. Large-scale reference cohorts such as ExAC/gnomAD are valuable for the population-frequency-based filtering of the myriad of detected sequence variants.

Objective: Using the example of fibrillinopathies, such as *FBN1*-related Marfan syndrome (MFS) and *FBN2*-related congenital contractural arachnodactyly (CCA), we assessed the frequency and co-occurrence of pathogenic *FBN1*/2 sequence variants in ExAC/gnomAD and the largest Swiss database of Marfan genomes, respectively.

Methods: By focusing on *a priori* pathogenic sequence variants in ExAC/gnomAD, we calculated conservative prevalence for MFS and CCA. In addition, we screened whole genomes (60× WGS, PE150) of ~550 patients with rare (aortic) disorders for pathogenic and functional sequence variants in *FBN1, FBN2*, and 12 pharmacogenes, respectively.

Results: We show the presence of pathogenic *FBN1/2* variants in the apparently healthy reference cohort ExAC/gnomAD, providing prevalence estimates for MFS and CCA. In our Swiss cohort, we identified two families with dual *FBN1* and *FBN2* mutations, explaining the variable phenotype within these families including clinical features of MFS and CCA. In one of these families, we also detected a pharmacogenetically actionable variant in a drug metabolizing enzyme.

Conclusions: Our results not only demonstrate that apparently healthy reference data sets may include individuals with late-onset or unrecognized disease, but also show that fibrillinopathies occur more frequently than expected and may co-occur. Furthermore, we emphasize the importance and increasing possibility of detecting digenic and pharmacogenetically relevant sequence variants using WGS.

Therapeutic Targeting of mTOR Pathway in a Severe Case of Progressive Osseous Heteroplasia

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Background: Progressive osseous heteroplasia (POH) is an ultra-rare genetic disorder characterized by an inactivating mutation in the GNAS gene that causes heterotopic ossification. Inhibition of mammalian target of rapamycin (mTOR) signaling pathway has been proposed as a therapy for progressive bone fibrodysplasia and non-genetic forms of bone heteroplasia. Herein we describe our experience with the use of Everolimus in the rescue therapy of an identical twin girl suffering an aggressive clinical phenotype of POH.

Methods: Clinical evaluation of the progression of the disease during Everolimus treatment was performed periodically. Cytokine markers involved in bone metabolism, as well as protein markers, related to the bone, activity was analyzed to explore bone turnover activity.

Results: The patient received Everolimus therapy for 36 weeks. During treatment, no clinical improvement of the disease was perceived. We could observe that biochemical parameters as bone turnover activity was significantly reduced, namely β-CTX ($r^2 = -0.576$, P-value = 0.016) and PNIP ($r^2 = -0.598$, P-value = 0.011). Additionally, bone metabolism biomarkers evidenced only a significant positive correlation with PTH levels.

Conclusions: Everolimus treatment did not modify the clinical progression of the disease in an aggressive form of POH, although an impact in the protein markers studied was observed.

Two Monochorionic Twins with a Critically Different Course of Progressive Osseous Heteroplasia

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Background: Progressive osseous heteroplasia (POH; OMIM 166350) is a rare autosomal-dominant genetic disorder characterized by progressive heterotopic ossification. POH is one of the clinical manifestations of an inactivating mutation in the *GNAS* gene. *GNAS* alleles show genetic imprinting, produce several transcript products, and the same mutation may lead to strikingly different phenotypes. The complexity of POH makes that this disorder still lacks therapeutic options.

Objectives: To describe a unique case of POH in two monochorionic twins, who presented an almost asymptomatic versus a severe clinical course.

Methods: The progression of the disease in both cases and the treatment effectivity were evaluated with measuring of serum markers involved in osseous metabolism. We report the results of the therapeutic interventions currently available for heterotopic ossification in the patient with a severe course.

Results: Due to the rapid progression of the disease in the patient with severe course a treatment protocol was administrated: mecasermin, naproxen, tretinoin, oral retinoid, pamidronate, itraconazole, methylprednisolone, and indomethacin. All the chosen interventions failed in stopping the formation of ectopic bone.

Conclusions: The difference in the clinical course between two twins with POH could be because of a very complex molecular mechanism that goes beyond a GNAS mutation. We failed at finding any medication that could ameliorate the symptoms of POH, let alone halting the progression of the disease.

Table 1. Treatments received by the patient with severe clinical course of POH.

DRUG	DOSE	MECHANISM	ADMINISTRATION LENGTH	ADVERSE EVENTS	CAUSE OF DISCONTINUATION	REFERENCES
Mecasermin	0.04 mg/day	rhIGF-I	15 days	No	Worse serum markers; same clinical	(35) Ueland T,2005 (36) Cao K, 2011 (37) Guntur AR, 2013
Naproxen	100mg	NSAIDs	40 days	Aphtous ulcers	Aphtous ulcers	*used as painkiller and antinflammatory in chronic conditions.
Topical Tretinoin	0.10%	Retinoid: Stimulation of Gsa expression at a transcriptional level	10 days	Red, swollen rash in the chosen regions	Ossification over the scapula grew	(43) Chan SD,1990 (44) Shimono K, 2010 (45) Shimono K, 2011
	0.025%					
Oral acitretin	10 mg/day		1 month	No	Coalescence of bony spikes of the back and progression of the plate over the left scapula, as well as appearance of new spikes surrounding the abdominal plates.	(46) MA Zasloff, 1998
Pamidronate	2.5 mg/kg	Bisphosphonate: Slows the release of calcium, blocking the mineralization of the bone matrix	3 days	Worsened myalgia and asthenia and onset of low-grade fever	Manifestations of POH progressed	(48) P Schuetz, 2005
Itraconazole	6.6 mg/kg/q.d.	Antifungal: acts as a potent suppressor of the Hh signaling pathway	3 months		Biochemical markers of bone formation returned to previous levels, and absence of clinical improvement in the disease progression	(54) J Kim, 2010
	9.5 mg/kg/q.d.		1 month			
Methylprednisolone	20 mg/kg/q.d.	Corticosteroid hormone	5 days		Absence of clinical improvement in the disease progression, despite reuction of markers of bone formation after the initial bolus.	(21) Pignolo RJ, 2011 (24)) Morales A, 2002
	Slow tapering		6 months			
Indomethacin	3 mg/kg/b.i.d	NSAIDs	6 months		Currently on indomethacin.	(64) Vanden Bossche, 2005 (68) Athanasou NA, 1994
	4 mg/kg/b.i.d					

From Diagnosis to Therapy: Novel Approach Reveals Celiprolol as Medical Therapy of Choice for Vascular Ehlers-Danlos Syndrome

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Background: Patients with the rare connective tissue disorder vascular Ehlers-Danlos syndrome (vEDS) are at increased risk for fatal aortic ruptures. Using a mouse vEDS model, we established an objective read-out system for the assessment of the clinically highly relevant biomechanical integrity of the thoracic aorta.

Objective: By means of this novel read-out system, we aimed to assess the effects of antihypertensive drugs on the biomechanical integrity of the weakened murine vEDS thoracic aorta as potential medical therapy in vEDS.

Method: Mice modelling vEDS were treated with the beta-blockers celiprolol (Selectol®) or bisoprolol (Bilol®) or the ARB losartan for 4 weeks. 1.5-mm-long sections of the ascending and descending murine thoracic aorta were mounted on a tissue puller and uniaxially stretched until rupture while recording the tensile force (in mN).

Results: The rupture force was significantly lower in untreated heterozygous compared to wild-type mice and decreased with increasing distance from the heart for both heterozygotes and wild-types. We showed that celiprolol but neither bisoprolol nor losartan increased the rupture force of the thoracic aorta in heterozygous mice (PMID: 31056650 and 31693161).

Conclusions: Our novel read-out system is suitable for detecting significant differences in the rupture force of the murine thoracic aorta and allows the assessment of the effect of candidate drugs on the biomechanical integrity of the aorta. Although the added value of other antihypertensive drugs in vEDS, if any, is unknown, celiprolol, but not losartan and bisoprolol, is currently the medical therapy of choice for vEDS, until further evidence emerges.

New Approach for Treating Hemophiliacs with Inhibitors Using Fc-Proteins and NK Cells

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Background: Fc fusion proteins are used in replacement therapy of genetic deficiency diseases to prolong half-life.

Objective: To determine if Fc-FVIII (used in the treatment of Hemophilia A) could interact with FcgR.

Methods and Results: Immune system cells bear Fc receptors. Fc-mediated interactions with these receptors, can lead to the activation or inhibition of function. We studied a panel of FDA-approved Fc-fusion proteins in terms of binding and activation of Fc-receptors. Using human Fc gamma receptor (FcγR) reporter cell lines, we demonstrated that Fc-FVIII bound and stimulated reporter cell lines expressing activating and inhibitory FcγRs (I, IIA, IIB, IIIA).

The finding that Fc-FVIII bound and activated cells via FcγRIIIA was studied in more detail. FcγRIIIA is expressed on most natural killer (NK) cells. We demonstrated that Fc-FVIIII activated NK cells as measured by IFNγ, perforin and granzyme B release; and killed a B cell clone bearing an anti-FVIII BCR. This killing was highly specific as B cells that did not express the anti-FVIII BCR were not killed. This may explain why Fc-FVIII has been reported to be more effective in tolerizing hemophilia A patients with inhibitors than unfused FVIII products. In future studies, we will explore the potential of Fc-proteins to target and kill unwanted B cells, especially in the context of protein replacement therapy and inhibitory antibody development.

Conclusions: Fc-FVIII can activate NK cells to kill anti-FVIII bearing B cells. This approach may be useful in preventing or treating patients undergoing protein replacement therapy with inhibitors.

RaDiCo (Rare Disease Cohorts): A Platform for Rare Disease (RD) E-Cohorts

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Background: Implementing RD cohorts is a challenge since RDs are seldom, often underdiagnosed and spread over the national territories.

Objective: To create a national platform dedicated to the development, within a research framework, of RD e-cohorts meeting strict criteria of excellence.

Material and Methods: The RaDiCo program coordinated by Inserm comprises an operational platform and several RD e-cohorts. The platform, built on the "cloud computing" principle, is oriented as an "Infrastructure as a Service"; Interoperable; In compliance with the General Data Protection Regulation; Within a Certified Health Data Host; Ensuring continuous monitoring of data quality and consistency; In line with the French Health Data Hub.

Results: 13 e-cohorts projects covering 67 RDs have been selected through a national call and launched (2017). Depending on cohorts, they aim at: Describing RDs' natural history; establishing phenotype-genotype correlations; Deciphering RDs' pathophysiology; Identifying new therapeutic avenues; Assessing RDs' societal and medico-economic impact*;* Identifying patients eligible for new therapeutic approaches. As of June 15, 2019, 4035 patients have been included and 1560 were eligible to come (recruitment target 97%); 26 publications appeared in international peer-reviewed journals.

Discussion: Several secondary objectives of the e-cohorts have been reached. Member of the 3rd RDs' National Plan, RaDiCo is tightly linked to patients' associations and advocacy groups. Partnerships with industry confirm RaDiCo's sustainability. The cohorts' PIs are involved in 10 European Reference Networks. RaDiCo participates to the RD-European Joint Program.

Conclusion: RaDiCo provides a flexible and easily sharable platform enabling the creation and follow-up of national/international RD e-cohorts.

The Rare Disease Cohorts (RaDiCo) Program: Set Up and Follow-Up of National and International E-Cohorts

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Background: Rare Disease (RD) professionals have highlighted the critical need to implement national/international, multidisciplinary, high-quality cohort studies to address key scientific and medico-economic questions.

Objective: To implement RD e-cohorts within a research framework, supported by an interoperable platform of mutualised expertise, resources and services, with shared standardised processes and tools.

Material and Methods: The RaDiCo program coordinated by Inserm has launched 13 national/ international e-cohorts, covering 67 RDs, selected through a national call. Depending on cohorts, they aim at: Describing RDs' natural history; establishing phenotype-genotype correlations; Deciphering RDs' pathophysiology; Identifying new therapeutic avenues; Assessing RDs' societal and medico-economic impact*;* Identifying patients eligible for new therapeutic approaches.

Results: The cohorts cover the following RDs: Congenital defects of the eye, Still`s disease, Low Phospholipid-Associated Cholelithiasis syndrome, Cystinosis, Alport syndrome, Skin RD burden, Genetics of Intellectual Deficiency and Autism Spectrum Disorders, Imprinting disorders, Mucopolysaccharidoses, Primary Ciliary Dyskinesia, Periodic Paralysis, Idiopathic Interstitial Pneumonia, and Vascular Ehlers-Danlos Syndrome. As of June 2019, 4035 patients have been included and 1560 were eligible to come (recruitment target 97%);

Discussion: RaDiCo assets are the following: A flexible, interoperable and easily sharable platform enabling the inclusion of new cohorts within an industrialization framework. Several secondary objectives of the cohorts have already been reached and published. RaDiCo cohorts are involved in 10 European Reference Networks and in the RD-European Joint Program. Sustainability is based on academic resources and several partnerships with industry.

Conclusion: RaDiCo developed e-cohorts and industrialized the process that enables including new RD cohorts either national or international.

Qualitative Research in Rare Disease; Methodologies for Conceptual Strength Regarding the Burden of Access

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Attempting to capture the experience of those navigating the United States health insurance system may seem intimidating. Each state has different resources, each program has different rules, and each person has a unique experience, often resulting in different outcomes. Within the rare disease space, this process has even more variation from person to person based on where they live, how knowledgeable they are, and what type of coverage they can access.

The authors attempted to address these issues and capture the general health insurance experience for families living with a specific rare disease through a novel methodology. The research team assembled a list of pre-determined challenges with input from parents of patients impacted by the disease, health insurance and physician experts. Respondents were assessed by disease subtype for health insurance literacy, the type of health insurance or other supportive services available, and then interviewed about their challenges with navigating the healthcare system. Interviews were conducted with a baseline cohort of 5 patients for each category, eliciting important concepts in an open-ended manner, collected using patient voice. Concepts were reviewed by independent coders, who grouped like-concepts, and added to the list any challenges provided by respondents that were not anticipated before the study. Subsequent cohorts of 5 patients in each category were recruited, and results were compared to the baseline cohort. If a new and important concept was raised, saturation had not been met, and an additional cohort was recruited for this category. Recruitment continued in this purposive manner until saturation had been met in each group.

This methodology shows what concepts were most important to patients when accessing coverage for treatments, medications, care, services, devices, and equipment needed for their rare disease. Recruiting to saturation of responses for each subgroup provided confidence that responses were representative of the larger population.

COLEC10 and 3MC Syndrome: Expanding the Genotypic and Phenotypic Spectrum of a Very Rare Disease

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Background: 3MC syndrome is an autosomal recessive disorder encompassing a variable spectrum of abnormalities, among which facial dysmorphisms are characteristic. Mutations in genes which encode proteins involved in the lectin complement pathway MASP1, COLEC11 and recently COLEC10 have been identified in patients with 3MC syndrome, supporting their key role during human development.

Objective: We present a 5 years old patient with typical 3MC phenotypic characteristics, including blepharophimosis, telecanthus, high arched eyebrows, fifth finger clinodactyly, horseshoe kidneys, diastasis recti, umbilical depression and sacral dimple. The diagnosis was confirmed by sequencing of COLEC10 gene and the putative pathogenic variant was functionally validated through in vitro assays.

Method: COLEC10 gene was analyzed through Sanger sequencing. The secreted protein CL-L1 was investigated in the plasma of the patient and her parents by Western blot. The variant was introduced by a site-specific mutagenesis approach into a plasmid encoding wild-type human CL-L1. HeLa cells were then transfected with the mutated or wild-type plasmid and culture supernatant evaluated in a migration assay.

Results: A homozygous frameshift variant c.807_810delCTGT p.(Cys270Serfs*33) was identified in the patient. Segregation studies confirmed the parents' carrier status for the variant. Functionally, the variant affects the chemo-attractive feature of CL-L1, as HeLa cells are less sensitive to the mutant protein compared to the WT one, resulting in a reduced migratory response.

Conclusion: We report a patient affected by 3MC syndrome who, besides typical phenotypic signs, presents a patent ductus arteriosus, never described in association to COLEC10 before. The variant causative role was functionally confirmed in an in vitro assay, where the mutated protein failed to act as a chemo-attractant. We thus provide further evidence for CL-L1 role during embryonic development.

Understanding Pathomechanism of Ultra-Rare Neuromuscular Genetic Disorder – GNE Myopathy

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Biological basis of pathogenesis of a large number of genetic disorders is not known, particularly for those diseases which affect the neuromuscular system. UDP-GlcNAc 2-epimerase /ManNAc kinase (GNE) is a bifunctional enzyme (N-terminal epimerase and C-terminal Kinase domain) that catalyzes rate limiting step in sialic acid biosynthesis. Homozygous misssense mutations in either epimerase or kinase domain of GNE leads to slowly progressive autosomal recessive genetic neuromuscular disorder, GNE Myopathy. These GNE related myopathies are characterized by hyposialylation of glycoproteins in muscle cells of patients and primary defect in either N or O-linked glycosylation. However, it appears from some recent experiments including those from our laboratory that mutant GNE may also affect targets that are not directly related to sialic acid biosynthesis. In particular cytoskeletal network, sarcomere organization and apoptotic signaling are likely to be altered in muscle cells. In absence of clear understanding of the pathomechanism, no treatment is currently available to cure the disease. Our laboratory focuses on deciphering alternate roles of GNE in regulating cell functions with an aim to identify more effective drug targets. We have established a HEK293 cell based assay system where pathologically relevant mutations of GNE are overexpressed along with GNE knockdown using shRNA. Also L6 rat skeletal muscle cell based model system with Gne single allele and reduced epimerase activity has been developed to affect actin organization. The system is validated by reduced sialic acid content of the cell and restoration of sialylation after supplementation with 5 mMsialic acid. Using this system, GNE has been shown to affect cell adhesion property via hyposialylation of β-1 integrin and altering G-actin and F-actin levels in GNE deficient cell lines. Mutation in GNE caused increased apoptosis via mitochondrial dysfunction that could be rescued by treatment with Insulin-like Growth Factor1 (IGF-1). Differential levels of ER resident Peroxiredoxin IV and upregulation of chaperones generate ER stress in absence of functional GNE. Role of HSP70 in regulating JNK signaling for stress induced apoptosis was determined to identify chaperone activator as effector molecule for treatment. Thus drug molecules regulating chaperone function can modulate protein misfolding and prevent protein aggregation observed in GNE myopathy. Our study clearly provides a base for understanding pathomechanism of GNE myopathy and the opportunity of using cell-based assays for diagnostics as well as exploring pharmacological drug molecules.

Tissue-specific Autoimmunity Controlled by Aire, a Gene Responsible for APECED

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Tissue-specific autoimmune diseases are assumed to arise through malfunction of two checkpoints for immune tolerance: defective elimination of autoreactive T-cells in the thymus, and activation of these T-cells by corresponding autoantigens in the periphery. However, evidence for this model and the outcome of such alterations in each or both of the tolerance mechanisms have not been sufficiently investigated. We studied these issues by expressing human AIRE (huAIRE) as a modifier of tolerance function in NOD mice wherein the defects of thymic and peripheral tolerance together cause type I diabetes (T1D). Additive huAIRE expression in the thymic stroma had no major impact on the production of diabetogenic T-cells in the thymus. In contrast, huAIRE expression in peripheral antigen-presenting cells (APCs) rendered the mice resistance to T1D, while maintaining other tissue-specific autoimmune response and Ab production against an exogenous protein Ag, due to the loss of Xcr1+ DCs, an essential component for activating diabetogenic T-cells in the periphery. These results contrast with our recent demonstration that huAIRE expression in both the thymic stroma and peripheral APCs resulted in the paradoxical development of muscle-specific autoimmunity. Our results reveal that tissue-specific autoimmunity is differentially controlled by a combination of thymic function and peripheral tolerance, which can be manipulated by expression of huAIRE/Aire in each or both of the tolerance mechanisms.

A Rare Case of Moyamoya in a Young Female

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Introduction:

Moyamoya refers to chronic progressive cerebrovascular (CV) diseases characterized by bilateral large intracranial artery stenosis or occlusion and development of prominent small vessel collaterals creating a smoky appearance on imaging. We hereby present a rare cause of a large stroke in a previously healthy young female hospitalized for gastrointestinal bleed (GI) bleed.

Case presentation:

Patient is a 35-year-old African-American female without any significant past medical history who presented to our hospital with generalized weakness and dark stools for 3 days. Initial physical exam was unremarkable except for heart rate of 106. Rectal examination revealed melanotic stool. Laboratory results were only significant for low hemoglobin of 4.9. Following routine resuscitation our patient underwent endoscopy which showed large 2 cm gastric ulcer with dark pigmented base and oozing edges. After endoscopic intervention with cauterization and vasopressin injection, hemoglobin remained stable above 8 during the rest of hospitalization. On second day of hospitalization, our patient developed right-sided facial droop, slurred speech, right upper and lower extremity weakness, and numbness. Right hemianopia was also noted. MRI and MRA of brain were immediately obtained and showed acute bilateral MCA infarcts in superficial an deep watershed areas, proximal right MCA occlusion, and left MCA artery segment. The vascular anomalies included leptomeningeal collaterals which were consistent with moyamoya disease (fig1&2). Patient was not a candidate for TPA as she had a recent GI bleed. Transthoracic echocardiogram with bubble studies did not show any thrombus, atrial or ventricular septal defect. Autoimmune and hypercoagulability studies were all normal.

Discussion:

Moyamoya is a rare condition commonly seen in East Asia with bimodal age onset peaking at 10 and 40. Our patient's presentation and imaging were consistent with moyamoya disease. Bilateral watershed pattern ischemia shown in MRI of brain is not a classic finding in embolic events. Hypoperfusion could potentially occur with low hemoglobin of 4.4 but should not have caused a CV accident in an otherwise healthy young patient. It is worth mentioning that our patient was never hypotensive. Our patient did not have any risk factors for vasculopathy, autoimmune diseases or thromboembolic events. Interestingly, she had never developed similar symptoms during her childhood. Our patient is from African American descent while moyamoya is commonly seen in East Asian population. She was referred to a tertiary care for external to internal carotid artery bypass as a preventive measure for future ischemic events.

Conclusion:

Moyamoya disease, although rare, should be considered as a differential for CV events in younger patients. Timely cerebral angiography and referral for external to internal carotid artery bypass can decrease the risk of debilitation in these rare cases.

Rare Disease in Organ Donors. 24-Mounthes Experience in Italy

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Introduction: Rare diseases (RD) are an heterogeneous group of pathologies that are not transmitted from organ donor to recipients. Nevertheless organs from these donors can presents functional deficit that could affect patient and/or graft survival. Aim of this work is to analize the incidence of rare disease in our organ donor population to support clinicians in the decision to use these organs.

Materials and Methods: We retrospectively assessed the incidence of RD in organ donors reported from July 2017 to June 2018, the risk attributed, the transplanted organs and the follow-up of the recipients.

Results: In 24 months, we had 19 donors (1%) affected by a RD (16 with a certain diagnosis, 3 with a suspected diagnosis). In 4 cases the donor hesitated in opposition, in 4 the risk attributed was unacceptable, in 1 standard, in 5 acceptable, in 2 negligible, in 3 a different risk was attributed depending on the organ considered. 4 donors were rejected by transplant centers, 8 were accepted with 18 transplanted organs (2 heart, 3 livers and 13 kidneys) in 17 patients. 3 recipients died for causes not related to MR, after a median follow-up of 9 months (range 4-18) 14 are alive with a functioning organ.

Conclusions: The evaluation is affected by the short times of the donation process which do not allow genetic or histological insights for each organ. Furthermore, the heterogeneity of RD and the unavailability of literature require a particular case-by-case evaluation. Therefore, a group of expert geneticists and transplant clinicians has been set up at the Superior Health Council to support surgeons in assessing the suitability of the organ, ensuring compliance with the relative safety and quality of the transplant.

Differential Gene Expression Patterns and Functional Analysis of Skin Samples from a Patient with Progressive Osseous Heteroplasia

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Background: Progressive osseous heteroplasia (POH) is an ultra-rare genetic disorder, characterized by progressive extra-skeletal bone formation. It is caused by heterozygous mutations in the *GNAS* gene, that is involved in multiple signaling transduction pathways and functions. We studied two monochorionic twin sisters of 7 years old diagnosed with POH, sharing the same *de novo* pathogenic mutation in *GNAS* but with very different clinical manifestations of the disease. While one of the sisters shows an aggressive and disabling disease, the other presents an asymptomatic phenotype.

Objective: We hypothesize that mechanisms controlling the gene expression might be under the different phenotypes showed by both affected twins. We analyzed gene expression patterns in skin samples from the affected twin to elucidate genes and pathways implicated in ectopic bone formation that could be used as potential therapeutic targets.

Method: We carried out a differential expression analysis followed by a functional analysis using 770 genes from 13 canonical signaling pathways in three affected skin samples from different locations compared with three skin samples from healthy subjects using nCounter technology (Nanostring).

Results: We found 24 differentially expressed genes comparing affected with normal skin, most of them under-regulated. We also detected different levels of gene expression dis-regulation between affected samples. Functional analysis revealed some pathways related to bone metabolism, like Wnt among others, under-regulation of G alpha (q) signaling events and serotonergic synapse, and up-regulation of genes implicated in epithelial-mesenchymal transition events.

Conclusion: We identified several genes and pathways implicated in the development of ectopic bone formation in POH.

GNAS Methylation Pattern and Epigenome Analysis in Two Monozygotic Twins with Progressive Osseus Heteroplasia but Different Clinical Phenotype

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Background: Progressive Osseus Heteroplasia (POH) is a rare genetic disorder characterized by progressive heterotopic ossification. It is caused by inactivating mutations of one of the most complex locus of the human genome, the GNAS gene, which shows genetic imprinting and encodes the α-subunit of the stimulatory G protein that plays a key role in several signaling pathways, including those related to bone formation.

Objective: Assuming that DNA methylation affecting gene expression can modify the phenotypic development of POH, the main objective of this study is to investigate the underlying clinical and molecular factors implicated in the unique case of a couple of monozygotic twins with the same GNAS inactivating mutation, but displaying a completely phenotypic discordance: the first twin develops an aggressive form of the disease while her sibling is almost asymptomatic.

Method: The global blood DNA Methylation patterns of both twins affected by POH was investigated through an epigenome whole association study (EWAS) using the microarray technology from Illumina (MethylationEPIC BeadChip; 850K). The evaluation of the methylation status of the complex locus GNAS1 was performed using the Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA).

Results: DNA methylation analysis showed some interesting differences in the methylation pattern in both twins that affect gene expression in several signaling pathways that could explain the phenotypic discordance of the present case.

Conclusion: Further studies on gene expression patterns in different tissues are necessary to completely investigate the differential onset of the disease.

New Approaches for Enzyme Replacement Therapy of Lysosomal Diseases with High Immunogenicity by Mass Spectrometric Identification of Antibody Epitopes

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Enzyme replacement therapies (ERT) have been successfully introduced for a number of Lysosomal Storage Diseases (LSDs) such as Gaucher (GD), Fabry (FD), and Pompe (PD) Disease. While effective for several LSDs, substantial problems can be caused by development of high immunogenicity. Patients who develop antibodies upon ERT can have allergic reactions, from mild symptoms to life threatening events. IgG antibodies may neutralize the lysosomal enzyme and prevent successful treatment. Here we report new therapeutic approaches by mass spectrometric identification and affinity characterization of antibody epitopes upon ERT, using synthetic epitope peptides that block antibodies directed against the infused enzyme. Therapeutic intervention using epitope peptide derivatives of low toxicity is expected to block neutralizing antibodies and substantially improve efficiency and safety of ERT. Identification of antibody epitopes from blood from FD and PD patients was obtained by a combination of proteolytic affinity- mass spectrometry and SPR biosensor analysis (SPR-MS). The epitope(s) from anti-alpha-galactosidase antibodies immobilized on a sepharose microcolumn were identified by trypsin digestion (2 hrs). The proteolytic peptide mixture was loaded onto an SPRMS interface; after washing out nonbinding peptides, the epitopes were eluted with 0.1 % TFA into the MS. The SPRMS combination was successfully applied to the epitope elucidation and affinity characterization of antibodies against alpha-galactosidase in 3 FD patients, and provided identical peptide sequences, αGal (309-332). The epitope (309-332) was synthesized by solid phase peptide synthesis (SPPS), and purified by reversed phase HPLC. SPR Analysis provided high affinity to the antibody (K_D , 39 nM). For cell culture studies skin fibroblasts are used to evaluate the epitope peptides on the uptake of lysosomal enzymes in the presence of the patient´s antibodies. The results showed that antibodies were blocked by tight binding to the epitope peptide, thus opening a new concept to reconstituting therapeutic efficiency of ERT.

In Vivo Rescue of α-Sarcoglycan Mutants by CFTR Correctors

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Sarcoglycanopathies are rare limb girdle muscular dystrophies in which the disruption of the sarcoglycan (SG) complex results in sarcolemma fragility and progressive muscle degeneration. Most of the reported cases are due to missense mutations originating a folding-defective sarcoglycan eliminated by the cells' quality control system, although potentially functional. The molecular mechanism of these diseases has been recently elucidated allowing to envisage novel therapeutic possibilities. To recover the mutants and avoid complex disruption, we exploited the use of protein folding correctors belonging to the CFTR modulators family.

Considering the exciting results obtained in vitro, we needed to generate novel and alternative animal models expressing a folding-defective SG, overcoming the unsuitability of the available mouse models of the disease. To this intent, we transduced with AAV-SGCA hind-limbs of α-SG null mouse pups resulting in "humanized hind-limbs" expressing the endogenous β-, γ-, δ-SG, and the human α-SG (wild type or mutated) carried by the virus. Histological and molecular characterization of the hind-limb muscles from 2 months old mice evidenced the recovery of the dystrophic phenotype when the wild type α-SG was transduced. On the contrary, the presence of a mutated α-SG resulted in pathologic features. Mice with "humanized hind-limbs" were then chronically treated with the most promising CFTR corrector, by intra peritoneal injection. First analyses revealed an increase of the α-SG content, a reduction of myopathy signs and the re-localization of the SG-complex at the sarcolemma in comparison to the vehicle treated animals. No sign of toxicity was observed during treatment.

Even though additional experiments are needed, this is the first in vivo evidence of the CFTR correctors efficacy in sarcoglycanopathy.

Development of Study Model for Rare Neural Disease Caused by Mutations in CYFIP2 Gene

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Background: West syndrome (WS) is an infantile neural disorder that causes a delay in development and infantile spasms. Mutations in the CYFIP2 gene were recently described as possible causes of WS. CYFIP2 has a role in actin polymerization through WRC, especially on dendritic spines and synaptosomes.

Objective: Establish a study model based on iPSC, through a non-invasive cell sample collected from a patient with CYFIP2 mutation and its reprogramming.

Methods: The urine sample was collected for a 2 years old Brazilian female patient characterized with the CYFIP2 - R87C variant. For isolation, the sample was washed, centrifuged, and the cellular pellet was cultivated until confluence. For reprogramming, cells were electroporated with episomal vectors and cultivated until reprogrammed colonies appeared. Colonies were labeled with pluripotent markers on days 15 and 24 to follow up.

Results: After isolation and expansion, based on its morphology and growth rate, cells were classified as type II urinary progenitor cells (UPC), according to the literature. After 15 days of reprogramming, pluripotent stem cells-like colonies can be visualized in culture. The TRA-1-60 and TRA-1-81 markers were even more evident in colonies on day 24, compared to day 15. On day 26 after reprogramming, colonies were manually harvested, and the cells were expanded until confluence.

Conclusions: The isolation and reprogramming of UPC collected from a patient with the CYFIP2 R87C variant were possible. Also, the reprogrammed cells expressed two of the pluripotent markers. The next step is the characterization of the reprogrammed cells, and differentiation into neural cells, making possible the study of the molecular mechanisms of CYFIP2 mutation and drug screening.

Editing of the CFTR Gene Mutation Causing Cystic Fibrosis in Pulmonary Epithelium Cells by CRISPR-Cas9

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BACKGROUND: Cystic fibrosis is a genetic, autosomal recessive disease caused by mutations in the CFTR gene (cystic fibrosis transmembrane conductance regulator). The most common mutation is F508del with around 70% prevalence worldwide. Currently, the disease has no cure and the main symptoms are treated. The technique of the CRISPR/Cas has favorable prospects to correct the mutation in DNA and rescue function of CFTR protein, improving patient's quality of life.

OBJECTIVE: The main objective this work was to build molecular tools to correct the F508del mutation of cystic fibrosis in a pulmonary epithelial cell line (CFBE).

METHOD: The work is divided into stages: (1) Design of sgRNA and construction of tools and (2) edition evaluation by qPCR, PCR and Sanger sequencing techniques.

RESULTS: The designs sgRNAs were selected based on the proximity of the mutation, number of off-targets and efficiency predicted. The insertion of the sgRNA sequence in the plasmid (px458) was confirmed by the PCR technique, digestion with restriction enzymes and sequencing. The transfection method selected was by lipid agents, showing an efficiency of about 9% in the entry of the plasmid into target cell. The built tool based ribonucleoprotein, had the RNA obtained by in vitro transcription. After formation of the complex with the purified protein (saCas9) this tool showed activity (30%) in recognizing and cleaving DNA in vitro. After 48 hours transfection cells, was made a sorting by flow cytometry and the correction confirmed by qPCR techniques, the detection the correction of the F508del mutation was below 12.5% or null, under the conditions tested.

CONCLUSION: New assays will be performed included design modifications for this tool to obtain satisfactory editing efficiency.

Outcomes of Appendiceal Carcinomatosis Patients Treated with Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy

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BACKGROUND. Mucinous appendiceal cancer (MAC) was historically classified as a colon cancer subtype and treated accordingly. However, emerging data show MAC to be a separate and heterogeneous group of malignancies with an incidence of 1.2 per 100,000 cases/year. Its common presentation with peritoneal carcinomatosis does not respond to conventional systemic chemotherapy. The unique approach of cytoreductive surgery with hyperthermic intraperitoneal chemotherapy (CRS/HIPEC) was developed for stage IV MAC treatment.

OBJECTIVE. To analyze outcomes after CRS/HIPEC in stage IV MAC.

METHODS. A prospective single-center CRS/HIPEC database was reviewed (February 1998-May 2020). Patient characteristics, perioperative variables, and overall survival (OS) after CRS/HIPEC were analyzed.

RESULTS. Of 537 MAC patients, 449 (84%) had completed and 88 (16%) had aborted CRS/HIPEC. Median age was 54 years with 63% females. Tumor subtypes included 54% low-grade (LG), 19% high-grade (HG), 19% high-grade with signet ring cells (HG-SRC), and 8% goblet-cell carcinoma (GCC). In completed CRS/HIPECs, median peritoneal cancer index was 27 with complete cytoreduction in 86%. Major morbidity and 100-day mortality rates were 22% and 1.6%. Median follow-up was 70 months. OS at 3, 5, 10, and 15 years was 75%, 66%, 53%, and 46%, respectively. Median OS was 131 months (CI95%: 108-154). Median OS by subtype was 196 (CI95%: 121-271), 66 (CI95%: 27-105), 31 (CI95%: 19-43), and 75 (CI95%: not available) months for LG, HG, HG-SRC, and GCC, respectively.

CONCLUSION. Stage IV MAC has a favorable prognosis when treated with CRS/HIPEC, regardless of subtype. Optimal outcomes are achieved with early referral to specialized CRS/HIPEC centers.

Peritoneal Dissemination from Ovarian Carcinosarcoma: Analysis of 14 Cases after Cytoreductive Surgery with Hyperthermic Intraperitoneal Chemotherapy

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BACKGROUND. Ovarian carcinosarcoma (OCS) is an uncommon and aggressive disease, accounting for only 1-4% of ovarian malignancies. Histopathology is unconventional as components arise from both epithelial and mesenchymal cellular lines. There is limited knowledge about the disease and treatment strategies are extrapolated from other more common gynecological malignancies. Clinical significance arises when anecdotal reports show 5-year survival rates under 20%.

OBJECTIVE. To evaluate the outcomes of cytoreductive surgery with hyperthermic intraperitoneal chemotherapy (CRS/HIPEC) in OCS patients.

METHODS. Patients undergoing CRS/HIPEC for peritoneal carcinomatosis from ovarian malignancies (1999-2020) were reviewed. Patients with confirmed histopathological diagnosis of FIGO stage III/IV OCS were identified.

RESULTS. Of 210 patients with ovarian malignancies, 17 (8.1%) had OCS. Fourteen (82.3%) of these received complete CRS/HIPEC, 9 patients (64.3%) treated for primary disease and 5 (35.7%) for recurrent disease. Histology for the carcinomatous component was 11 (78.6%) high-grade serous, 1 clear-cell and 2 undifferentiated adenocarcinomas. The sarcomatous components were 8 (57.1%) heterologous, 4 (28.6%) homologous and 2 (14.3%) undifferentiated sarcomas. Median age at surgery was 63 years (IQR: 53.7–74.0). Seven (50.0%) patients had FIGO stage IV disease. Clinical and intraoperative characteristics were similar to other ovarian malignancies. Median follow-up was 20.9 months (CI95%: 14.9-26.8). Median overall survival was 23.9 months (CI95%: 18.6-29.3) with a 3-year survival rate of 33.8%.

CONCLUSIONS. CRS/HIPEC shows promising survival outcomes in the treatment of an advanced stage rare ovarian malignancy. Further collaborative studies with larger sample sizes and longer follow-up may elucidate the role of CRS/HIPEC in ovarian carcinosarcoma.

Phosphorylated Glucocerebrosidase: Next-Generation, Cost Effective ERT for Gaucher Disease

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Background: Existing ERTs for GD type 1 are insufficiently effective for alleviating cardiac, bone and lung symptoms. Neither are they adequate to treat neuronopathic forms of GD because the enzymes do not cross the BBB, and even if they would, are insufficiently targeting neuronal cells.

Objective: Oxyrane aims to produce a more stable β-glucocerebrosidase enzyme rich in M6P that targets a broader cell-type population, thereby alleviating remaining unmet needs in GD type 1 and unmet needs in GD type 2/3. Oxyrane employs 2 administration routes: intravenous approach to treat systemic symptoms of GD type 1, and intracerebroventricular approach to treat both neurological and systemic symptoms of GD type 2/3.

Method: Oxyrane uses yeast-based technology to augment the phosphorylation of N-glycans independently of the enzyme's 3D structure. Consequently, the OxyGCase variant is rich in M6P and shows a 50-fold increased M6P-mediated uptake in neuronal cells compared to imiglucerase.

Results: Studies in a D409V KI mouse model show that OxyGCase is efficiently targeting brain cells in various brain regions after intracerebroventricular administration, shown by enhanced activity levels and increased substrate reduction. Similar results are observed in peripheral organs, both via intravenous and intracerebroventricular administration. OxyGCase outperforms imiglucerase with respect to activity levels and substrate reduction, both via the intravenous and intracerebroventricular approach.

Conclusion: OxyGCase was proven to be efficacious (preclinical POC), safe and tolerable (6-month NHP toxicology study). Clinical POC of OxyGCase is planned for both administration routes. In addition, attractive Cost of Goods allow for a significant sale price reduction as next-generation GD type 1 treatment.

Clinical Evolution of an Intermediate Type 2-3 Gaucher Patient

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Aim: The presentation of the clinical evolution for a Gaucher Disease patient, with genetic double mutation [D409H; H255Q], classified as intermediate type 2-3 GD

Background: Gaucher disease (GD) is an autosomal recessive lysosomal storage disease, which is mainly due to mutations in the GBA gene. Most of the mutant alleles described so far bear a single mutation. However, there are a few alleles bearing two or more DNA changes. It has been reported that patients homozygous for the [D409H;H255Q] (p.Asp448His:p.His294Gln) double mutant allele, present a severe type 2 neurologic Gaucher disease.

Case report: Our case is a 5 year-old boy. His parents have Albanian origin without consanguinity. Pregnancy and delivery were normal. Birth weight was 2.9 kg. At the age of 4 months, he was referred to our service because of hepatosplenomegaly and thrombocytopenia**.** At the age of 7 months, enzyme activity of glucocerebrosidase resulted very low and the level of chitotriosidase was high, confirming the diagnosis of Gaucher disease. Genetic testing identified the presence of homozygote double mutation [D409H;H255Q]. Enzyme replacement therapy with imiglucerase, every other week has been started. At the age of 15 months, this boy presented neurological signs, including neck rigidity and ocular movement disorders. The neurological signs became more evident and during the next visit, at the age of 20 months, the child presented: generalized dystonia, swallowing difficulties, chest deformity (Fig.1), oculomotor apraxia, extrapyramidal syndrome, coordination impairment. During the follow up period, the child showed good improvements of visceral and hematological signs, together with the stability of chitotriosidase and lysoGB1 biomarkers, until the age of 3 years old. During the fourth year of life, the level of lysoGB1 were rising (Fig.3). At this time, we started ambroxol-chaperone therapy. During last two years, the neurological status of the child was complicated by hydrocephalus (Fig.2) and seizures. Due to swallowing difficulties, we carried out a percutaneous gastrostomy, which preceded a ventriculo-peritoneal shunt, performed one week later. Neurological impairment remains catastrophic, despite the treatment with ERT, ambroxol, levetiracetam and baclofen.

Discussion: A double mutation [D409;H255Q] is present in approximately 50 % of Albanian GD patients. Most of our patients have a heterozygous form, combined with N370S (type1 GD) and rarely combined with L444P or F213I, leading to neuropathic forms, with a variable gravity. Homozygous form leads to severe neurological impairments, such as type 2 GD [Michelakakis et al., 2006], dying within the first or second year of life, meanwhile another study reported a case, such as an intermediate phenotype 2-3 [Filocamo et al., 2005], surprisingly of Albanian origin. Another publication [Swati Sathe et al 2008] describes another Albanian patient, with double mutation [D409;H455Q], who died at the age of 31 months, presenting neurological impairment and hydrocephalus. Our case presented neurological signs, before the age of two years old. Initially we thought for Gaucher type 2 but he continues to survive, for three more years. Genotype-phenotype correlations completed the criteria, for an intermediate form 2-3 Gaucher disease type. According to the literature [Ellen Sindrasky et al.,2010] some patients with intermediate form 2-3, present severe neurovisceral manifestations, during infancy or early childhood but they survive past the second year of life, with death occurring in mid childhood (age 3-7 years old). Under the treatment with ERT, our patient has had a slow evolution till the age of 3 years old. Now the patient is 5 years old and alive, despite severe neurological impairment. This case is another example that GD is a continuum of phenotype.

Conclusion: Intermediate type 2-3 Gaucher disease presents a slower clinical evolution compared to the type 2, nevertheless the neurological impairment remains life threatening, despite the treatment.