The International Working Group on Gaucher Disease

1st IWGGD SYMPOSIUM

Hosted by Pr. Hans Aerts, Leiden Institute of Chemistry, Leiden University (The Netherlands)

8 - 11 MAY 2022

ABSTRACT BOOK

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The International Working Group on Gaucher Disease

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SYMPOSIUM

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IGA VOLUNTEER PROGRAMME: BUILDING AN INTERNATIONAL PATIENT GROUP: ACHIEVING A GLOBAL REACH WITH LIMITED RESOURCES

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BACKGROUND: The overall goal of the volunteer programme (VP) is to support the work of IGA and enable us to meet the diverse needs of our global community.

AIMS: Through increasing the numbers of volunteers, IGA is gathering more experience, perspectives from different countries, cultures, new ideas, and ways how to improve the quality of life of families with Gaucher disease. The programme was developed to provide a structure and invest in the volunteers through providing training and supervision.

METHODS: The VP has its own strategic plan, in accordance with the IGA strategic plan. We have volunteer job descriptions, timesheets, recognition, and mentors or project leads to support volunteers. All volunteers were interviewed and went through orientation, and they have regular meetings with the volunteer coordinator. Volunteers are involved in the improvement of the VP through feedback and evaluation questionnaires. Additional training and workshops are available for volunteers.

RESULTS: In 2021 and 2022 IGA has had support from 41 volunteers, from 34 countries, in 11 projects, and around 1400 volunteer hours. Most of the volunteers (around 70%) were recruited through social media. The Regional Manager Programme and the GARDIAN Champions program are completely driven by volunteers.

CONCLUSIONS: The Volunteer Programme is an established system to recruit and retain volunteers. It provides future recruitment of board members. It enables the IGA to increase impact through the realization of more projects. Board members can focus more on strategy and leading the organization forward. Expanded community support and increased skill set are noticeable.

DISCLOSURE FOR ALL AUTHORS: The IGA received unrestricted grants in 2021 for specific projects from Avrobio, M6P, Freeline, Oxyrane, Pfizer, Orphazyme, Gain, Prevail, Sanofi, and Takeda. The CEO of the IGA has received fees for consultancy work and speaker fees from Akcea, Oxyrane, Prevail, Sanofi Genzyme, and Takeda. The Projects Officer has received speaker fees from Sanofi and Takeda.

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EXPERTS VIEWS ON COVID-19 VACCINATION AND THE IMPACT OF THE PANDEMIC ON GAUCHER DISEASE PATIENTS

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BACKGROUND: The COVID-19 pandemic carries high morbidity and mortality in individuals with chronic disorders, and introduced unanticipated challenges for patients with rare diseases.

AIMS: With worldwide vaccination developments, we aimed to evaluate Gaucher disease (GD) experts’ views on COVID-19 vaccines, and understand the impact of disease on GD patients.

METHODS: A group of GD experts was assembled to perform a crosssectional, descriptive survey using an online questionnaire distributed between January and April 2021 amongst a convenience sample of GD experts worldwide.

RESULTS: We collected responses from 19 GD experts from ten countries, treating a total of 1417 GD patients, mostly GD type 1 (GD1). All responders support or strongly support COVID-19 vaccination for GD patients. Of the patients who started new treatment during the pandemic, 42.5% (95% CI 28%-57%) were given oral substrate reduction therapy (SRT) and 59% of the patients that changed their enzyme replacement therapy (ERT) regimen switched to SRT. Of 82 GD1 patients who contracted COVID-19, 77 (93.9%) were either asymptomatic or mildly symptomatic.

CONCLUSIONS: GD experts support COVID-19 vaccination for their patients. The majority of GD patients infected with COVID-19 had a mild course, suggesting that the risk and severity of COVID-19 in patients with GD is like the general population.

DISCLOSURE FOR ALL AUTHORS: Ian J. Cohen has received honoraria for scientific talks, grants and advice from: Sanofi-Genzyme, Protalix, Pfizer and Takeda-Shire. Noa Ruhrman-Shahar has received honoraria for scientific talks, grants and advice from: Sanofi-Genzyme, Pfizer and Takeda-Shire. Tova Hershkovitz has received honoraria for
scientific talks, grants and advice from: Sanofi-Genzyme, Pfizer and Takeda-Shire.
Claus Niederau has received honoraria for scientific talks, grants and advice from: Abbvie, Alexion, Biogen, Falk, Sanofi-Genzyme, Gilead, MSD and Takeda-Shire.
The S2MC Gaucher Unit receives support from Sanofi/Genzyme for participation in the ICGG Registry, from Takeda for the GOS Registry and Pfizer for TALIAS. The Unit also receives research grants from Takeda, Pfizer, Sanofi-Genzyme and Centogene. Ari Zimran receives honoraria from Takeda, Pfizer, Sanofi and BioEvents and consultancy fees from Takeda, AVOBIO, Insightec, Todos Medical and Prevail therapeutics. Shoshana Revel-Vilk receives grant/research support, honoraria and advisory fee from Takeda, Pfizer and Sanofi-Genzyme.
Maria Domenica Cappellini is a member of the advisory board for: BMS/Celgene, Sanofi-Genzyme, Novonordisk, Vertex/CRISPR and Vifor.
Hagit Baris Feldman has received honoraria for scientific talks, grants, and consults from: Sanofi-Genzyme, Protalix, Pfizer, and Takeda-Shire. She serves on scientific advisory boards of Sanofi-Genzyme and Igentify and in the past also in Shire and Regeneron.
The remaining authors declare that they have no competing interests.

10 PROJECT SEARCHLIGHT STUDY METHODOLOGY: REAL-WORLD EVALUATION AND VALIDATION OF A RARE DISEASE ALGORITHM TO IDENTIFY PERSONS AT RISK OF GAUCHER DISEASE IN THE USA

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BACKGROUND: Misdiagnosis and long diagnostic odysseys underpin significant morbidity in patients with rare diseases such as Gaucher disease (GD) and acid sphingomyelinase deficiency (ASMD). Using information from a de-identified US Electronic Health Record database of >100M individuals, two patient identification models have been developed to detect individuals at risk of GD.

Machine-learning methods using characteristics indicative of GD curated from the literature and individual patient diagnoses, laboratory tests, symptom onset, and demographics resulted in two models based on age of occurrence or feature prevalence.

AIMS: We are conducting a prospective study to evaluate these GD models in real-world settings by three iterative steps.

METHODS: Step I will deploy the models in the clinical data infrastructures of participating US health systems, rank individuals, and deliver a composite ranking score for each patient. Step 2 comprises chart review of the top 50 highly ranked patients at each site and referral of appropriate patients to Step 3 for consent and confirmatory diagnostic testing. Enzyme assays for GD and ASMD, and biomarkers glucosylsphingosine and lyso-sphingomyelin will be performed in parallel due to overlapping symptomatology. GBA or SMPD1 sequencing will be performed as appropriate.

RESULTS: Primary objectives include estimating the diagnostic yield of the models and identifying the magnitude of unrecognized GD in highly ranked patients. Secondary objectives include describing characteristics of all highly ranked individuals by presence of GD diagnosis, assessing the distribution of ranking scores and their correlation to diagnostic yield. Exploratory objectives include describing the relationship between the ranking score and diagnostic yield; assessing the diagnostic yield for ASMD among patients highly ranked for GD and describing the characteristics of persons highly ranked for GD but positive for ASMD.

CONCLUSIONS: The study should demonstrate an effective new paradigm for identifying patients with undiagnosed rare diseases. This study is sponsored by Sanofi.

DISCLOSURE FOR ALL AUTHORS: Lisa Sniderman King MSc CGC, Mario Aguiar MD, Alexandra Dumitriu PhD, Patrick Pavlick BS, and Amanda Wilson PhD are employees of Sanofi, Cambridge, MA, USA, and may hold shares and/or stock options in the company.
Alexandra Chiorean MSc and Martin Montmerle MBA are employees of Quinten Health, Paris, France
Pramod Mistry MBBS – Has received grant support, honoraria, and travel reimbursement from Sanofi
François Modave PhD – Has received honoraria from Sanofi Neal Weinreb MD – Shire HGT: Consultancy, Honoraria, Speakers Bureau; Sanofi: Consultancy, Honoraria, Membership on an entity’s Board of Directors or advisory committees, Research Funding, Speakers Bureau; Pfizer Corporation: Consultancy, Honoraria, Membership on an entity’s Board of Directors or advisory committees, Speakers Bureau.
This work was previously presented at the 18th Annual WORLD Symposium®, Feb 7 - Feb 11, 2022, San Diego, CA, USA.

36 SELF-MANAGEMENT OF GAUCHER DISEASE

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BACKGROUND: Individuals diagnosed with Gaucher Disease (GD) are living with it 24/7 yet 99% of their time they are not in direct contact with a healthcare professional. They have to manage not only their disease but its impact on their life in all aspects including psychological and behavioral in other words self-management of their life and disease. For many of them without treatment, this is the only means to deal with GD. In more common diseases self-management led to significant decrease in burden of disease and improvement in quality of life.

AIMS: Aim is to define self-management, highlight its role for optimum care of GD patients and how it can be implemented for the best outcome.

METHODS: We are one of the working groups of the IWGGD and IGA to write patient centric guidelines for management of GD. We conducted an extensive search of the literature on self-management and related concepts in relation to rare diseases. We had several Zoom meetings to discuss the role of self-management in optimizing care for patients, and prepared a draft guideline for GD practice.

RESULTS: Self-management is defined as the tasks that individuals must undertake to live well with one or more chronic conditions. Optimal self-management is vital for an optimal quality of life and optimal effectiveness of medical care. Fundamental is partnering as clinician with the patient as an expert on their own life and body, and as the expert in many settings outside the consultation room. Sharing knowledge and information is the basis for broader empowerment and self-management support. Family members have their own burden of GD as well as their own role in disease management.

CONCLUSIONS: The guideline is ready to present and explain. The general overview is being finalized, so it can be sent in for review and publication.

DISCLOSURE FOR ALL AUTHORS: The authors do not have any conflicts of interest.

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IGA/IWGGD JOINT PROJECT ON HOME ENZYME REPLACEMENT THERAPY


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BACKGROUND: In many countries, especially in the Western world, patients have the possibility to receive enzyme replacement therapy (ERT) at home, to the satisfaction of all involved. Nevertheless, there are still many countries where home ERT is still not available or acceptable by regulators and physicians. The Covid-19 pandemic affecting hospital delivery of ERT around the world, led the IGA to investigate with its global community the current provision of home therapy and how home therapy is organized in each country.

AIMS: The IGA, together with the IWGGD, aims to provide guidelines and perhaps other deliverables to facilitate a proper home infusion therapy provision, especially in countries where home ERT is not available.

METHODS: A review of relevant literature is being prepared. An exploratory survey is held under the representatives of the member organizations of the International Gaucher Alliance. A thorough field consultation will be performed.

RESULTS: At the IWGGD conference, preliminary outcomes from the literature review and the exploratory survey under member representatives will be presented and discussed.

CONCLUSIONS: We hope to provide greater clarity on the importance and benefits of home infusion therapy in an attempt to make it a standard of care for patients with Gaucher disease on ERT.

DISCLOSURE FOR ALL AUTHORS: The authors do not have any conflicts of interest.

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PATIENTS’ VIEW ON GENE THERAPY DEVELOPMENT IN GAUCHER DISEASE, FABRY DISEASE AND MUCOPOLYSACCHARIDOSIS TYPE III: A QUALITATIVE STUDY

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**BACKGROUND:** Several new treatment modalities are being developed for lysosomal storage disorders (LSDs), including gene therapy. As the currently available treatment options and their influence on disease progression differ greatly within the spectrum of LSDs, willingness to undergo gene therapy might differ among patients with different LSDs and/or their representatives. The width of the LSD spectrum is illustrated by the differences between type 1 Gaucher disease, Fabry disease and Mucopolysaccharidosis type III (MPSIII). For type 1 Gaucher and Fabry disease several therapies are available resulting in a near normal and improved but individually varying prognosis, respectively, while no treatment options are available for MPSIII.

**AIMS:** To identify factors influencing patients’ and/or their representatives’ decisions whether or not to undergo gene therapy.

**METHODS:** Focus group discussions and semi-structured interviews were conducted with patients with type 1 Gaucher disease, Fabry disease and MPSIII. Parents of MPSIII patients were also included as patients’ representatives.

**RESULTS:** Nine Gaucher patients, 23 Fabry patients, two adult MPSIII patients and five parents of MPSIII patients participated in the study. The five main themes that arose were: outcome of gene therapy, risks and side effects, burden of gene therapy treatment, current situation and ethical aspects. Participants’ views ranged from hesitation to eagerness to undergo gene therapy. Severe disease and limited treatment options or effectiveness augmented the willingness to undergo gene therapy: Gaucher and Fabry patients deemed the burden of treatment important. Fabry and MPSIII patients and parents ranked outcome high, suggesting hope for improved outcomes of gene therapy. Gaucher patients ranked outcome low, which could indicate a more cautious attitude towards gene therapy.

**CONCLUSIONS:** This study underlines the importance of exploring patients’ needs and expectations before deploying limited resources for the development of therapies for patient groups of which a significant subset may not be willing to undergo that specific therapy.

**DISCLOSURE FOR ALL AUTHORS:** EE is as a sub-investigator involved in a pre-marketing study with Sanofi Genzyme. ML and CH are involved in premarketing studies with Sanofi-Genzyme, Protalix and Idorsia. MB was a sub-investigator in the Lysogene genetherapy study for MPSIII (NCT02053064). All other authors declare to have no disclosures.

**SESSION 2: LABORATORY: GENETICS & BIOCHEMISTRY**

**53 GAUCHER DISEASE DIAGNOSIS USING LYSO-GB1 ON DRY BLOOD SPOT SAMPLES: TIME TO CHANGE THE PARADIGM?**

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**BACKGROUND:** Gaucher disease (GD), caused by recessive mutations in GBA and a concomitant reduction of lysosomal β-glucocerebrosidase (GCase) activity, leads to glucocerebroside storage in various tissues and a multi-system disease known for its great phenotypic heterogeneity. The gold standard for diagnosing GD is detecting reduced GCase activity in peripheral blood cells combined with GBA mutation analysis. The use of dried blood spot (DBS) specimens for diagnosis offers many advantages, including easy collection, the need for a small amount of blood, and simpler transportation. However, DBS has limitations for measuring GCase activity.

**METHODS:** In this cross-sectional study, we publish seven years of experience using DBS samples and plasma levels of the deacylated form of glucocerebroside, glucosylsphingosine (Lyso-Gb1) for GD diagnosis.

**RESULTS:** Of 444 screened subjects, 99 (22.3%) were diagnosed with GD at a median [range] age of 21 (1-78) years; 59 diagnosed with GD type 1 (GD1) and mild GBA variants [i.e., N370S (c.1226A>G) homozygous, combined N370S (c.1226A>G) /R496H (c.1604G)] were significantly older, 25 (2-78) years, than 30 patients diagnosed with GD1 and severe GBA variants [i.e., N370S (c.1226A>G) combined with another variant], 11 (2-35) years, and nine children diagnosed with neuronopathic GD (nGD), 2 [1-11] years (p<0.001). The median [range] Lyso-Gb1 levels for genetically confirmed GD patients vs. controls subjects were 252 (9-1340) ng/ml and 5.4 (1.5-16) ng/ml, respectively. Patients diagnosed with GD1 and mild GBA variants had lower Lyso-Gb1, 194 (9-1050) ng/ml, than those diagnosed with GD1 and severe GBA variants, 447 (38-1340) ng/ml, and those diagnosed with nGD, 325 (116-1270) ng/ml (p=0.001). Carriers of GBA variant had higher Lyso-Gb1 levels, 5.8 [2.5-15.3] ng/ml, compared to wild type GBA, 4.9 [1.5-16], ng/ml (p=0.001).

**CONCLUSIONS:** Our study confirms the use of Lyso-Gb1 sampling from DBS as a screening test for GD. GBA gene sequencing is important for confirmation of diagnosis and genetic consultation.

**DISCLOSURE FOR ALL AUTHORS:** T.D, M.B.-C., and M.I. have no conflict of interest to declare. The SZMC Gaucher Unit receives support from Sanofi/Genzyme for participation in the ICGG Registry, from Takeda for the GOS Registry, and Pfizer for TALIAS. The Unit also receives research grants from Takeda, Pfizer, Sanofi/Genzyme, and Centogene. P.B., C.B., G.K., C.C., and M.I.I. are employees of Centogene GmbH. A.R. is the founder and was the CEO of Centogene GmbH during the study. A.Z. receives honoraria from Takeda, Pfizer, and BioEvents and consultancy fees from Takeda, NLC pharma, Insightec, and Prevail therapeutics. S.R.-V. receives grant/research support,
CONCLUSIONS: We developed and validated a method of combined analysis of the biomarkers Lyso-Gb1 and Lyso-Gb3 in DBS by LC-MSMS that will enable a faster and accurate diagnosis.

DISCLOSURE FOR ALL AUTHORS: The study was partly sponsored by Takeda Belgium. François Eyskens received travel grants and fees attaining adboards or giving presentations by Sanofi, Takeda, Amicus, Biomarin and Acthelion. Amber Van Baelen has nothing to disclose.

55 MARKERS OF INFLAMMATION AND ALPHA DEGRANULATION DEFECT OF PLATELETS IN PATIENTS WITH GAUCHER DISEASE SHOW


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BACKGROUND: Flow cytometry enables studies of the activation state of circulating platelets and the reactivity of circulating platelets in response to various platelet agonists and has several advantages over light aggregometry. Most importantly for the present study, platelets of patients with thrombocytopenia can also be accurately analyzed.

METHODS: We studied unstimulated and stimulated activation markers on platelets, i.e., aIIbb3 integrin (PAC1), P-selectin (CD62P), and lysosomal-associated membrane protein (LAMP3/CD63), in 194 patients with Gaucher disease (GD). To determine whether levels of platelet activation markers were altered in vivo (i.e., without ex vivo stimulation), we studied the surface activation of these markers on unstimulated platelets.
RESULTS: Patients with GD had a higher expression of CD63, median 8.12% (range 0.20%-63%), compared to the reference range (p<0.001). Splenectomized GD patients had higher expression of PAC1 and P-selectin, 9% (0.75%-81.6%) and 2.1% (0.11%-71.53%), compared to those with intact spleen, 1.9% (0.02%-41.66%) and 0.42% (0.01%-14.53%) (p<0.001). To determine the capacity of platelets to respond to stimulation, we tested platelet reactivity in response to ex vivo agonist stimulation. Reduced platelet reactivity (-2 SD of reference range) was found in 104 (53%, 95% CI 46%-61%) patients, of whom 16 (8.25%, 95% CI 4.8%-13%) had more severe platelet dysfunction. In a multivariate model, only lyso-Gb1 levels were associated with the more severe platelet dysfunction. Platelet dysfunction was also found in patients receiving many years of GD-specific therapy. Sixty-one (46%) of 131 adult patients who completed the bleeding tendency questionnaire reported positive bleeding history. In a multivariate logistic model, older age (OR (95% CI), 1.07 (1.2-1.12)) and low P-selectin reactivity (OR (95% CI), 2.04 (1.25-3.46)) were associated bleeding tendency.

CONCLUSIONS: We recommend adding platelet flow cytometry to the assessment before interventional procedures. Further studies are planned to understand the degradation defect and the in vivo increased CD63 expression on platelets of patients with GD.

DISCLOSURE FOR ALL AUTHORS: The SZMC Gaucher Unit receives support from Sanofi/Genzyme for participation in the ICGG Registry, from Takeda for the GOS Registry, and Pfizer for TALIAS. The Unit also receives research grants from Takeda, Pfizer, Sanofi/Genzyme, and Centogene. S.R.-V. receives honoraria and research funding from Takeda, Pfizer, Sanofi/Genzyme. C.C. is an employee of Centogene GmbH. M.N., D.F., T.D., M.I., M.B.-C. and E.B. have no conflict of interest to declare. A.Z. receives honoraria from Takeda, Pfizer, and BioEvents and consultancy fees from Takeda and Prevail Therapeutics. This study was sponsored by a grant from Pfizer.

5 SCLEROSTIN AND DICKKOPF-RELATED PROTEIN-1 IN BONE MANIFESTATIONS OF GAUCHER DISEASE

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BACKGROUND: Patients with Gaucher disease (GD) have progressive bone involvement that clinically presents with debilitating bone pain, structural bone changes, bone marrow infiltration (BMI), Erlenmeyer flask deformity (EM), and osteonecrosis. The Wnt signaling pathway and its inhibitors (sclerostin (SOST) and Dickkopf-related protein-1 (DKK-1) have a primary role in the regulation of bone metabolism.

AIMS: This study investigates the role of Wnt antagonists, SOST, and DKK-1 in GD-related bone disease.

METHODS: This cross-sectional study included 36 patients with GD and 20 healthy controls. The patients were classified into different cohorts based on the presence and the severity of bone manifestations, including pain, bone structural changes, density abnormalities, and bone marrow infiltration (BMI). A Brief Pain Inventory (BPI) was utilized to assess pain. The serum levels of SOST, DKK-1 were quantified by ELISAs.

RESULTS: Significantly increased levels of SOST were found in the GD cohort presenting with pain, BMI, and EM deformity. The multiparameter analysis demonstrated that 95% of GD patients with bone pain, bone marrow infiltration, and EM deformity had increased levels of SOST. The majority of patients with high levels of SOST also have osteopenia or osteoporosis. A Pearson linear correlation analysis demonstrated a positive correlation between serum DKK-1 and SOST in both healthy controls and GD patients with normal bone density. However, the balance between SOST and DKK-1 waned in GD patients with osteopenia or osteoporosis.

CONCLUSIONS: The osteocyte marker, SOST, when elevated, is associated with bone pain, bone marrow infiltration, and EM flask deformity. The altered SOST and DKK-1 ratio correlate with the reduction of bone mineral density. These data confirm that the Wnts signaling pathway plays a role in GD-associated bone disease.

DISCLOSURE FOR ALL AUTHORS: MMI contracted research: Takeda. JD, HK, LNI, AF: Nothing to disclose. OGA advisory boards, consulting, research contracts, speakers fee: 4DMT, Amicus, Avrobio, Sangamo, Sanofi, Takeda; Freeline, Genentech, Protalix, Sangamo.

6 STUDY OF miRNA EXPRESSION PROFILES IN GAUCHER PATIENTS AND THEIR RELATIONSHIP WITH THE SEVERITY OF BONE INVOLVEMENT

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BACKGROUND: Bone manifestations are one of the most prevalent comorbidities in Gaucher disease (GD) and, compromise the quality of life. They are evaluated by DEXA and MRI and several scores are available to assess its severity. Nowadays, no good biomarkers are available for this complication. miRNA are small non-coding RNA molecules whose function is regulate gene expression, and several of them were related to bone disease.

AIMS: Identify different miRNA expression patterns, depending on the severity of bone involvement in patients with GD and, evaluate its predictive value.
METHODS: 60 GD naïve patients, were selected and classified according to their bone disease severity using the S-MRI score, considering mild bone disease (MiBD; S-MRI<5), moderate (MoBD; S-MRI: 5-11) and, severe (SB; S-MRI>11). miRNA was obtained from plasmatic exosomes using miRCURY Exosome Serum/Plasma and miRNeasy Serum/Plasma Advanced kits (QIAGEN) following manufacturer instructions. NGS technology and principal component analysis, corrected by sex and age, were applied to determine miRNA expression profiles.

RESULTS: Patients were distributed proportionally in each group (20-20-20), gender distribution was equal between groups, and age was [median (Q1-Q3)]: 19.0 (4.00-40.00) – 40.5 (28.25-56.00) – 37.5 (31.25-47.00) years for MiBD, MoBD and SBD respectively. When compared MiBD against MoBD 3 miRNA were downexpressed in the MoBD group meanwhile 1 was increased. When MiBS vs SBD were compared 4 miRNA were downregulated and 5 increased on the SBD group. Only one miRNA is upregulated on MoDB and even more so in the SBD.

CONCLUSIONS: One miRNA, related inversely with osteoblastic differentiation is increased according with the severity of bone disease. So, those preliminary results show an altered miRNA expression depending on the bone severity. Nowadays, we are validating these results and trying to predict target-genesis and the biological pathway they are involve.

DISCLOSURE FOR ALL AUTHORS: Project founded by a Sanofi’s grant (SGZ-2019-12810).

8 ASSESSMENT OF LYSOSOMAL ACID LIPESE ACTIVITY AS BONE MINERAL DENSITY BIOMARKER IN GAUCHER DISEASE TYPE 1 PATIENTS

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BACKGROUND: Bone disease is one of the most severe complications in Gaucher disease type 1 (GD1). Previously projects were focus on identification of specific bone-related biomarkers, whereas it did achieve variable results. Lysosomal acid lipase (LAL) deficiency leads a rare dyslipidemia with high cholesterol and triglycerides’ levels, triggering atherosclerosis among other manifestations. Loss of bone mineral density (BMD) is related to high cholesterol levels and atherosclerosis. Recently, LAL reduced activity was linked to dysfunction in osteoblastogenesis in murine model.

AIMS: To assess the relationship between LAL activity and the BMD in GD1 patients at diagnosis.

METHODS: GD1 patients with BMD evaluation and blood sample, were selected. We measured LAL activity by fluorometric methods using the specific inhibitor Lalstat-2. The BMD was assessed, at lumbar spine, by dual energy x-ray absorptiometry analyzing z and t scores. Quantitative variables were described as median(25%-75%), qualitative as frequencies, and U Mann-Whitney and Rho Spearman were applied to evaluate the relationship between LAL and BMD.

RESULTS: Forty GD1 patients were analyzed. Age, gender distribution and BMD is showed in Table 1. Most of the GD1 patients are females and all the osteoporotic subjects are pre-menopausal females. LAL activity did not allow classify BMD in GD1 patients (p=0.5), or statistical correlation with BMD, t or z scores (p=0.26).

CONCLUSIONS: Our results does not support the use of LAL activity as diagnosis biomarker to stratify the BMD status in GD1 patients. Further investigations, increasing the number of patients and the use of LAL activity as follow-up BMD biomarker will be develop.

DISCLOSURE FOR ALL AUTHORS: Nothing to disclose.

Table 1. Age, gender, t and z score for each group

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Gender</th>
<th>t score</th>
<th>z score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>20</td>
<td>0.23</td>
<td>0.34</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>40</td>
<td>-0.95</td>
<td>-0.74</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>50</td>
<td>-1.29</td>
<td>-1.02</td>
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76 GLYCOSPHINGOLIPIDS DRIVE NEUROINFLAMMATION INVOLVING MICROGLIA-NK CELL-ASTROCYTE ACTIVATION IN NEURODEGENERATION DUE TO GBA DEFICIENCY

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BACKGROUND: Biallelic mutations in GBA1 in Gaucher disease (GD) underlie defective acid β-glucosidase and the accumulation of bioactive lipids, glucosylceramide (GlucCer) and glucosylsphingosine (GlcsPh) that underlie the diverse disease manifestations. Mutations in GBA1 and resulting
aberrant glycosphingolipid metabolism in neurodegenerative diseases is a major focus of translational clinical investigations. In the present study, we investigated the functional significance of Gba in individual cell lineages by targeted rescue of Gba in Gbαn1/Inl mice. Using FACS, single cell RNAseq and lipidomic approaches we showed that neuronal injury in neuronopathic GD (nGD) brain was found to be associated with microglial activation and attrition of homeostatic microglia, concomitant with infiltration of peripheral immune cells as the disease advanced. The immune cell infiltrate was notably diverse but strikingly enriched for GZMA+ lymphocytes in both genetic nGD mouse models as well as in chemically induced nGD mice brain. Further evidence of key role of tissue macrophages in driving disease pathology was probed in long term monitoring of mice having Gba deficiency in tissue resident macrophages. These mice with accumulated GlcCer and GlcSph in the brain, show age related neurodegeneration indicated by clinical signs and elevated neurofilament light (NF-L) levels, accompanied by neuro-immune inflammation. Treatment of Gba deficient mice with brain permeant UGCG inhibitor G2161 lead to reduction in brain GlcCer and GlcSph bioactive lipids, reduced serum biomarkers of neurodegeneration (NF-L) and abrogated aberrant immune cell activation involving microglia and NK cells while significantly improving survival. In conclusion our study delineates the role of tissue macrophages and immune cell involvement in Gba-associated neurodegeneration.

SESSION 3: GAUCHER DISEASE CLINICAL SPECTRUM

16 OVERLAPPING AND DIVERGENT HEPATIC AND LIPOPROTEIN PHENOTYPES IN UNTREATED ADULTS WITH GAUCHER DISEASE VERSUS UNTREATED ADULTS WITH ACID SPHINGOMYELINASE DEFICIENCY FROM TWO PIVOTAL CLINICAL TRIALS

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BACKGROUND: Gaucher disease (GD) and acid sphingomyelinase deficiency (ASMD, historically known as Niemann-Pick disease types A and B) are rare autosomal recessive lysosomal storage diseases with overlapping clinical features but distinct pathophysiology due to biallelic pathogenic variants in GBA and SMID1 that cause toxic accumulation of glucocerebroside and sphingomyelin, respectively. Frequent misdiagnoses contribute to diagnostic odysseys while patients develop serious complications.

AIMS: To compare hepatic and lipoprotein phenotypes in GD1 and ASMD and correlations with organomegaly.

METHODS: We compared baseline hepatic and lipoprotein phenotypes descriptively (mean±SD) in untreated adults in Sanofi-sponsored-placebo-controlled trials: ENGAGE (eliglustat for GD1) (NCT00891202,N=40) and ASCEND (olipudase-alfa for non-neuronopathic-ASMD) (NCT02004691,N=36).

RESULTS: Mean age was 31.8Y in GD1 patients and 34.8Y in ASMD patients. Both populations had moderate splenomegaly (multiples of normal, MN): 13.20±5.91 (GD1) versus 11.45±4.36 (ASMD) and moderate hepatomegaly (MN): 1.40±0.32 (GD1) versus 1.53±0.42 (ASMD). Platelet count (x10^9/L) in GD1 was 76.76±18.68 compared to 111.38±31.77 in ASMD. Liver function tests were normal in GD1 unlike those in ASMD: aspartate aminotransferase (U/L): 28.15±8.55 (GD1) versus 42.86±31.91 (ASMD); alanine aminotransferase (U/L): 25.55±11.56 (GD1) versus 42.72±29.23 (ASMD); total bilirubin (mg/dL): 0.77±0.26 (GD1) versus 1.12±0.96 (ASMD). Lipoprotein pattern (in mg/dL) was strikingly divergent, except for low HDL cholesterol in both disorders: triglycerides: 149.2±72.88 (GD1) versus 194.88±81.47 (ASMD), total cholesterol: 123.98±26.18 (GD1) versus 197.33±42.73 (ASMD), LDL cholesterol: 68.85±22.53 (GD1) versus 145.86±49.80 (ASMD), HDL cholesterol: 26.25±8.08 (GD1) versus 22.23±9.14 (ASMD). Additional markers are presented in the Figure. In both populations, Pearson correlation coefficients showed moderate inverse correlations between HDL and organ volumes: spleen volume: r=-0.63 (GD1) versus -0.60 (ASMD) and liver volume: r=-0.50 (GD1) versus -0.45 (ASMD).

CONCLUSIONS: In untreated adults, liver dysfunction and atheregenic lipoprotein profiles were more pronounced in ASMD than GD1. Awareness of distinct lipoprotein phenotypes will enhance clinical care, inform investigations of disease mechanisms and yield new biomarkers.

DISCLOSURE FOR ALL AUTHORS: PKM: Principal investigator in the Sanofi sponsored ENGAGE trial; Consulting fees and honoraria from Sanofi. DC: Has received consulting fees from Sanofi. SAI: Principal investigator in the Sanofi sponsored ASCEND trial; Consulting fees and honoraria from Sanofi. RL: Principal investigator in the Sanofi sponsored ASCEND trial; Consulting fees and honoraria from Sanofi EL: Principal investigator in the Sanofi sponsored ENGAGE trial;
Consulting fees and honoraria from Sanofi. CEP: Has received consulting fees from Sanofi. BLT: Former Sanofi employee, owns Sanofi stock; currently an independent consultant, has received consulting fees from Sanofi. MPW: Principal investigator in the Sanofi sponsored ASCEND trial. Has received research support, travel reimbursement, and consulting fees from Sanofi. MCF: Sanofi employee, owns Sanofi stock RMP: Sanofi employee, affiliated with MCPHS University MR: Sanofi employee, affiliated with MCPHS University LHU: Sanofi employee, owns Sanofi stock

Note: This abstract is an adaptation of a poster presentation from WORLDSymposium 2022

ADVANTAGES OF DIGITAL TECHNOLOGY IN THE ASSESSMENT OF BONE INVOLVEMENT IN GAUCHER DISEASE

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BACKGROUND: Digital transformation and image transmission techniques is one of the most important challenges in today’s healthcare environment, requiring the implementation of improvements and the search for solutions aimed at guaranteeing the correct management of healthcare data improving culture of personalized and precision medicine.

AIMS: To apply artificial intelligence techniques to a structured reporting model of bone involvement in our cohort of Spanish patients with Gaucher disease (GD).

METHODS: A total of 419 MRI bone marrow studies performed in 130 GD patients containing radiological data and clinical and analytical parameters. The information in each patient’s clinical report was also analyzed, including demographic, genetic, age, gender, biomarkers, age to start therapy and the accumulated years of exposure to it data. Applying random forest supervised machine learning to try to obtain data markers to predict the evolution of bone disease.

RESULTS: 68 males and 62 females diagnosed with GD were included, following the protocol defined as S-MRI from April 1995 to December 2021. The same radiologist with expertise in bone marrow MRI has evaluated all exams and applied the structured report template in order to proceed to the analysis of the affection as objectively as possible.

The model defines with accuracy that the degree of infiltration by S-MRI and the age to start therapy have location the most importance to predict the evolution of bone disease. Other variables involved are the characteristics and location of the infiltration and age at diagnosis and genotype.

CONCLUSIONS: In this study we obtain information that the artificial intelligence methods could be an accurately tool to predict the bone disease complications in GD patients.

DISCLOSURE FOR ALL AUTHORS: I am submitting this abstract with the intent of attending the IWGGD 2022 Scientific meeting and presenting. I confirm each co-author has been informed of this abstract submission and has agreed to all information as it was submitted.

INVESTIGATING STRUCTURAL BRAIN CHANGES IN GAUCHER DISEASE TYPE 1: A MAGNETIC RESONANCE IMAGING STUDY
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BACKGROUND: Gaucher Disease (GD) is an autosomal recessive metabolic disorder due to glucocerebrosidase deficit. Three main clinical phenotypes were identified: GD1, a non-neuropathic form; GD2, an acute neuropathic form and GD3, characterized by a slower and progressive neurological involvement.

AIMS: Although the pathogenesis of GD is well known, a thorough description of structural brain changes, such as cortical complexity (cortical thickness and gyrification) in GD patients, especially type I, has not been provided yet.

METHODS: In the present study, 16 GD1 (mean age = 43 years, SD = 14 years) and 16 healthy controls (HC) without any neurological and psychiatric disease (mean = 44 years, SD = 14 years), underwent a 3T Magnetic Resonance Imaging session including T1 scans. A Region of Interest (ROI) based morphometry analysis using CAT12 was performed to investigate focal brain differences between GD1 and HC.

RESULTS: First, results showed that the disease duration significantly predicted a grey matter loss in GD1 patients (R2=0.63, F(1,14)=24.09; p< .001). Second, An independent t-test on cortical thickness (CT), between GD1 and HC showed that regions in prefrontal (left: 46, IFSa, 8C, s6-8; right: 46, p9-46, 8Av, s6-8, 6ma, FEF), posterior cingulate (l-v23ab) and parietal cortex (r-V3b) were significantly reduced (p< .05, uncorrected) in GD1 than HC (figure 1A). Concurrently, CT in the temporal region r-VMV3 was higher in GD1 patients than HC. Also, the cortical gyrification (CG) in a region of the secondary auditory cortex (l-LBelt) was significantly reduced in GD1 than HC (p<.01, uncorrected) whereas the CG in prefrontal (r-8Av) and frontal regions (r-p47r), cingulate cortex (l-23d, l-v23ab), and inferior temporal gyrus (l-PH) were significantly reduced in HC than GD1 patients (p<.01, uncorrected) (figure 1B).

CONCLUSIONS: Overall, the focal brain differences found between GD1 patients and HC may predict and clarify the cognitive impairments and behavioural alterations observed in this category of patients.

DISCLOSURE FOR ALL AUTHORS: I confirm that disclosures of all co-authors were declared.

SESSION 4: NEW DEVELOPMENTS AND YOUNG RESEARCHERS

11 miRNA EXPRESSION PROFILE IN A NEW MODEL OF GBA KNOCK-OUT OSTEOSTROBLASTS

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BACKGROUND: Up to 90% of Gaucher Disease (GD) patients report bone symptoms. Even though the molecular basis of bone involvement in GD is unclear, the role of osteoblasts and the possible role of miRNAs in bone pathogenic process have been demonstrated.

AIMS: Developing a model of GD osteoblasts, characterizing the differential miRNA profile of wild-type (wt) and GBA knock-out (KO) cells, exploring the role of the most promising differentially expressed miRNAs in bone pathogenesis.

METHODS: CRISPR/Cas9 technology was used to develop GBA KO cell, which were characterized by assessing beta-glucosidase (GCase) expression and activity, and glucosylsphingosine (Glcsph) accumulation. Alizarin Red, Sirius Red, and qRT-PCR were used to analyse osteoblast features. miRNA expression was assessed by NGS and qRT-PCR.

RESULTS: We edited a human osteosarcoma cell line (SaOS) obtaining a GBA KO clone displaying 1% of residual GCase activity and accumulating Glcsph.. Compared with wt cells, GBA KO osteoblasts displayed decreased alkaline phosphatase (ALP) and collagen type I (COL1A1) mRNA expression, decreased calcium and collagen deposition, increased receptor activator of NF-kB ligand/osteoprotegerin expression ratio, and no differences in ALP activity.

Then, we studied the miRNA profile of these cells using NGS, identifying up- and down-regulated candidates to be further investigated. Among the upregulated miRNAs in GBA KO cells, we further validated miR-488-3p, as its expression and secretion reached 14-fold and 4-fold increase, respectively, in comparison to wt. To assess the possible role of this miRNA on bone pathogenesis, we transfected miR-488-3p on SaOS wt and we noticed decreased ALP and COL1A1 mRNA expression.

CONCLUSIONS: We developed and characterized a new model of GD osteoblasts, performed a miRNA expression profile, and identified miR-488-3p as a possible player in bone pathogenesis of GD.
DISCLOSURE FOR ALL AUTHORS: EP: Sanofi-Genzyme fellowship; MS and AD: Sanofi-Genzyme and Takeda speaker fees, travel grants, research grants.

Graphical Abstract

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BIOMIMETIC 3D TISSUE PRINTING PROVIDES “PERSONALIZED” IN VITRO BONE MODEL IN GAUCHER DISEASE (GD)

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BACKGROUND: The utilization of live cells encapsulated in biomaterials (hydrogels) as bioinks enables the use of 3D printing for tissue engineering. The applications of this novel technology include creating organ-level structures such as bone and cartilage and in vivo-like in vitro models for preclinical drug screening. Skeletal disease is the leading cause of morbidity and reduced quality of life in GD.

AIMS: To engineer bone tissue and simulate bone microenvironment in GD using 3D biomimetic printing.

METHODS: Patients with GD were grouped into two cohorts as mild vs. severe bone disease. Peripheral blood mononuclear cells (PBMCs) from patients with GD were co-cultured with human bone marrow-derived mesenchymal stem cells (hMSCs) in different ratios, and differentiated into osteoclasts and osteoblasts respectively. GCase irreversible inhibitor, CBE, was used as a positive control to simulate the GD microenvironment. After the formation of osteogenic aggregates in the form of spheroids that mimic the morphology and physiology of native bone tissue, spheroids were then bioprinted to form a rectangular tissue patch.

RESULTS: Histology and immunostainings at 4-weeks revealed positive alkaline phosphatase and tartrate-resistant alkaline phosphatase (TRAP) stainings with multinucleated cells in the co-cultured model, demonstrating the efficacy of the model and corroborating with the gene expression studies. There were no substantial changes in differentiation to osteoblasts. However, H&E stainings demonstrated significant morphological deformities, which were more pronounced in the “severe” cohort.

CONCLUSIONS: The bioprinted bone tissue as a first human model provides the cellular basis of the developmental bone abnormalities in GD. This technique has the potential to be adapted to personalized medicine not only for individualized drug screening but also as an approach to bone regeneration.

DISCLOSURE FOR ALL AUTHORS: OGA – advisory boards and consulting fees: 4DMT, Amicus, Avrobio, Sangamo, Sanofi Genzyme and Takeda; research contracts: 4DMT, Amicus, Avrobio, Freeline, Genentech, Protalix, Sangamo, Sanofi Genzyme and Takeda; speaker’s bureau: Sanofi Genzyme and Takeda. MI – consulting fees: Takeda. DB, RPL, MHK, NC, and ITO: Nothing to disclose:
- The clinical protocol was approved by the ethics committees and data protection agencies at all participating sites (Western Institutional Review Board, WIRB # 20131424).
- Partial funding: Investigator-Initiated Award from Shire Pharmaceuticals USA, a member of the Takeda group of companies IIR-USA-002657.

A 3D model for investigating bone disorders in Ga

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VERTEBRAL OSTEONECROSIS AND ASSOCIATED DEFORMITY IN GAUCHER DISEASE

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BACKGROUND: Osteonecrosis (ON) of the large joints of the appendicular skeleton is well described in Gaucher disease. ON of the axial skeleton is less recognised. The Gauchereite Project is a National UK database including approximately 90% of known Gaucher patients, n=250.

METHODS: We reviewed available MR- and plain radiographic images of the vertebral column of the cohort: demographic information was captured in 2017. The most...
Recent images dated between 2013 and 2019 and were analysed for the presence of ON, Fracture and H-shaped endplate deformity (defined by the presence of rectilinear deformities on both end plates). Fracture was determined by DC using standard morphometric analysis. ON and endplate deformities were determined by PD, based on 20 years’ experience and specific radiologic training by SMcD. Demographic parameters were as recently published.1


RESULTS: 224 patients had images suitable for analysis. In total, 2294 segments (vertebrae and sacral segments from T10 to S3) were characterised. Of 224 patients, 24 had at least one segment of ON, 24 had at least one fracture and 12 had H-shaped endplate deformity. Of 2294 segments analysed, 99 had ON, 50 had fracture and 22 had H-shaped deformity. There was considerable overlap between these abnormalities. Splenectomy was associated with ON (Chi square test, p<0.0001), Fracture (Chi square, p<0.0001) and H-shaped deformity (Fisher’s Exact test, p<0.0001). The interval between symptom onset and treatment initiation was associated with ON (Mann-Whitney, p=0.0003), but not fracture (p=0.056) or H-shaped deformity (p=0.11).

CONCLUSIONS: Here we describe the under-recognised prevalence and associations of ON and related deformity in the vertebral column in Gaucher disease. As with ON at other sites, splenectomy and delay in initiation of specific therapy are powerful risk factors for ON of the vertebral column.

Distribution of ON, fracture and H-shape deformity

22 MONOCYTE IN VITRO MODEL OF GAUCHER DISEASE: EVALUATION OF THE EFFECT OF THERAPIES ON INFLAMMATION AND OSTEOCLASTOGENESIS

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BACKGROUND: Skeletal alterations in Gaucher patients are not completely resolved by specific therapies. Moreover, bone mineral density pathophysiology is not completely understood. Research studies revealed that both bone formation and resorption could be altered and play a role in
bone pathology. Our group has developed a macrophage model of Gaucher disease by specifically knocking-out GBA1 gene in THP-1 cell line, using CRISPR/Cas9 technology (THP-1 GBA1-KO). Edited cells showed reduced glucocerebrosidase (GCase) protein expression and activity, along with the increased level of glucosylsphingosine (GlcSph).

AIMS: We aim to further characterize the proinflammatory cytokines release and osteoclastogenesis in this GBA1 KO model and to study effect of different specific treatments.

METHODS: THP-1 wild type and GBA1-KO were grown in culture and exposed to the following treatments: recombinant human GCase (imiglucerase, ERT), eliglustat (substrate reduction therapy, SRT), ambroxol (chaperone) or Pentosan-polysulfate (PPS, anti-inflammatory), GCase activity, GlcSph, IL-1β and TNF-α levels and the tendency of monocytes to differentiate into osteoclasts were analyzed.

RESULTS: THP-1 GBA1-KO cells displayed increased levels of both proinflammatory cytokines and osteoclast differentiation.

GCase activity was detected only in cells treated with ERT. GlcSph accumulation was reduced, not only ERT and SRT treatments, as expected, but also in cells treated with Ambroxol that promoted GlcSph release to the extracellular media. Levels of cytokines were modulated by all the treatments at different levels. Osteoclastogenesis was reduced by all the treatments.

CONCLUSIONS: The THP-1 GCase KO model recapitulates most features of GD monocytes. All evaluated treatments were able to ameliorate, at least in part the pathological phenotype, as assessed by GlcSph levels, proinflammatory cytokines production and osteoclast differentiation.

DISCLOSURE FOR ALL AUTHORS: PR received consulting honoraria, travel and research grants from Takeda AD received consulting honoraria and travel grants from Takeda and Sanofi EP received a fellowship from Sanofi.

3 MODELING MISFOLDING OF HUMAN DISEASE-CAUSING LYSOSOMAL ENZYMES IN DROSOPHILA MELANOGASTER AS MEANS TO TEST THERAPEUTIC MODALITIES

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2The Future Scientists Center–alpha Program At Tel Aviv Youth University - Tel Aviv (Israel)

BACKGROUND: Lysosomal storage disorders result from substrate accumulation, due to decreased activity of lysosomal enzymes. Some of these proteins are recognized as misfolded in the ER, which leads to their retention, to their ER Associated Degradation (ERAD) and to activation of the Unfolded Protein Response (UPR). UPR can be alleviated by chaperones, small molecules, with the ability to cross the blood brain barrier, which facilitate exit of the mutant proteins from the ER, thus allowing their trafficking to the lysosomes.

AIMS: We use Drosophila melanogaster as a platform to assess UPR induced by mutant lysosomal enzymes and to follow the efficacy of chaperones in decreasing the ER stress.

METHODS: In the present study we tested the ability of arimoclomol, a chemical chaperone which stabilizes the interaction of Heat Shock Factor 1 (HSF1) with Heat Shock Elements (HSEs), thus leading to increased expression of heat shock proteins and better folding of misfolded proteins, to reduce neurodegeneration caused by misfolded glucocerebrosidase (GCase). We also compared arimoclomol to the pharmacological chaperone ambroxol.

RESULTS: Arimoclomol increased the number of brain dopaminergic cells, improved climbing ability, decreased neuroinflammation and increased the life span of flies expressing mutant GBA1 similar to the effect of ambroxol. We have also created flies expressing mutant acid sphingomyelinase (ASM) variants, associated with Nieman-Pick types A/B. Interestingly, our analysis showed no significant stress caused by the mutant ASM variants and thus no significant UPR activation or neurodegeneration in the flies. Therefore, there was no phenotypic rescue by arimoclomol due to apparent minimal misfolding in this specific disease.

CONCLUSIONS: Our results strongly indicate that arimoclomol is an efficient chemical chaperone for mutant GBA1 variants.

DISCLOSURE FOR ALL AUTHORS: None

1 EXPRESSION OF CRISPR INDUCED L444P MUTATION IN THE GBA1b ORTHOLOG OF DROSOPHILA MELANOGASTER

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BACKGROUND: The fruit fly Drosophila melanogaster has two GBA1 orthologs: GBA1a and GBA1b. GBA1b encodes bona fide glucocerebrosidase (GCase) while GBA1a directs the expression of a protein that regulates differentiation-dependent apoptosis, which plays a role in the larval midgut regression. There are two existing fly lines with mutations in these orthologs, that resulted from the insertion of the Minos transposable element in the GBA1 orthologs. The insertions lead to premature termination of the open reading frame, which caused a 133 C-terminal amino acids deletion in the GBA1b protein and a 33 C-terminal amino deletion in the GBA1a encoded protein. Two other labs also created deletion mutations in the two fly Drosophila orthologs.
AIMS: In the present research, we aimed at generating mutant Gba1b harboring human mutation.

METHODS: In an effort to generate mutant GBA1b orthologs which will harbor the two most prevalent Gaucher-disease mutations: L444P and N370S, the CRISPR-Cas9 editing technology was used.

RESULTS: While Leucine at position 444 in mature human GCase is conserved in the fly, Asparagine 370 in humans is Aspartic acid in the fly. Surprisingly, flies homozygous for the D370S mutation presented very low GCase activity and therefore were abandoned. Flies homozygous for the L444P mutation presented low activity, however, they presented a low substrate accumulation, with activation of the Unfolded Protein Response and inflammation. The neurodegeneration in the flies as tested by their negative geotaxis was minimal and was reflected by a minor effect on the survival of the flies. Ambroxol had a minor effect on UPR, inflammation, motor activity, or survival of the GBA1bL444P/L444P mutant flies.

CONCLUSIONS: Taken together our results indicate that the severity of the L444P mutation in the flies is different from its known effect in GD patients.

DISCLOSURE FOR ALL AUTHORS: None

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TRANSGLUCOSYLATION OF GLYCERS BY GLUCOCEREBROSIDASE AND GBA2: A LABORATORY FINDING

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BACKGROUND: Glucocerebrosidase 1 and 2 (GBA1 and GBA2) have been discovered to transglucosylate specific metabolites like cholesterol. Transglucosylation implies the enzymatic transfer of a glucose from a glucoside donor to an acceptor metabolite. We are presently examining whether more such ‘glucolites’ exist. Potential candidates in this respect are glycerols (diacylglycerol and monoacylglycerol).

AIMS: To investigate whether human GBA1 and GBA2 are able to glucosylate diacylglycerol or monoacylglycerol via a transglucosylation reaction.

METHODS: We employed NBD-diacylglycerol (NBD-DAG) and NBD-monoacylglycerol (NBD-MAG) to investigate their transglucosylation by human GBA1 and GBA2 using 4MU-β-glucose or glucosylceramide as glucoside-donors. In tandem, we tested glucosylceramide synthase (GCS) in its ability to glucosylate these metabolites using UDP-glucose. Product formation was monitored by HPTLC.

RESULTS: GBA1 was able to transglucosylate NBD-DAG and NBD-MAG. This activity was also observed for GBA2, but not for GCS. GBA1 likely also degrades glucosylated diacylglycerols and monoacylglycerols, as pointed out by kinetics data. Investigations are currently ongoing to examine formation and function of these metabolites in Gaucher materials.

CONCLUSIONS: Human GBA1 and GBA2 can transglucosylate diacylglycerols and monoacylglycerols in vitro. In addition, GBA1 is able to degrade the glucosylated structures again, which may prove relevant for patients with Gaucher disease.

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ABILITY OF GLUCOCEREBROSIDASE TO HYDROLYZE 6-O-ACYLATED β-GLUCOSIDES AND SYNTHETIC ANALOGUES

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BACKGROUND: Glucocerebrosidase (GBA) is a retaining β-glucosidase able to degrade glucosylceramide (GlcCer) in vivo and artificial fluorogenic substrate 4-Methylumbelliferone-β-glucose (4MU-β-glc) in vitro. More recently its ability to metabolize cholesteryl-β-glucose has been recognized. In plants steryl-β-glucosides (sterolins) occur and some of these are also present as 6-O-acylated structures.

AIMS: Study the ability of human GBA to hydrolyze 6-O-acylated 4MU-β-glucose and close analogues.

METHODS: We synthesized 6-O-acylated 4MU-β-glc and close analogues (1-5) by replacing the ester linkage by diverse bioisosters (Figure 1). Activity of recombinant human GBA towards these new fluorogenic substrates was assessed by detection of release of 4-MU, and potential transglucosylation was monitored by HPTLC and LC-MS/MS.

RESULTS: Recombinant GBA was found to be able to hydrolyze diverse 6-O-acylated 4MU-β-glucosides and to be able to also transfer the 6-O-acylated glucosides from these substrates to cholesterol in a transglucosylation reaction as revealed by HPTLC analysis. We observed that when this reaction was performed in cell lysates the ester bond in 6-O-acylated glucosides is not completely stable, and ~10% of glucose is released after 24 h incubation probably due to the presence of esterases. Analogous artificial fluorogenic substrates where the ester bond was replaced by more stable bioisoster linkages were synthesized and examined. Substrate 4 with an ether-linked alkyl at C6 was found to be stable, rendering stable transglucosylation products.

CONCLUSIONS: Human glucocerebrosidase tolerates specific modifications at C6. The generated substrate 4MU-β-glucose with an alkyl moiety linked via an ether bond to C6 seems specific for GBA since it is not hydrolyzed by GBA2. Of note, GBA is able to metabolize 6-O-acyl β-glucosyl-sterols naturally occurring in plants.
MyGauch™ - Web and Mobile Technology Platform for Gaucher Disease Patients’ Care


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BACKGROUND: Globally, we see an increase in the desire of patients to play an active role in their healthcare management. In rare diseases, such as Gaucher Disease (GD), patient engagement through a combined digital technology may have added importance. It is recommended that patients with GD be managed by experts having long-term experience with the diverse clinical manifestations and potential GD-related or unrelated co-morbidities; their follow-up visits at the center of excellence are usually on an annual or semi-annual basis. However, disease-related and un-related medical events may occur in-between these visits, some of which may be emergent. Furthermore, patients with GD need a life-long commitment to their therapy; adherence to the therapy regimen is essential to achieve normalization. The use of patient-reported outcome measures (PROMs) is fundamental to ensure that what matters to patients is captured in a valid, reliable, responsive, and feasible manner.

METHODS: MyGauch™ provides digital tools for self-management, treatment reminders, adherence, and report of PROMs. Moreover, patients get pain and fatigue monitoring and provide physical activity and nutrition data. An Alert-Dashboard enables the clinic to be alerted to changes in the patient status. An app-mail enables accessible communication between the patient and the health professionals on issues of concern to patients.

INTEGRA™, by LifeOnKey, a platform for Integrated, is the backbone of MyGauch™. Its security and data privacy complies with GDPR guidelines, and it is HIPAA compliant.

A mobile app (Figure 1-2) was developed for the use of the patients.

CONCLUSIONS: To prove patients’ satisfaction from their Gaucher treatment while using MyGauch™, we started in February 2022 an investigator sponsored study (ISS) for adults with GD. All data is captured in a GD-specific PatientHub™ by LifeOnKey.

DISCLOSURE FOR ALL AUTHORS: The SZMC Gaucher Unit receives support from Sanofi/Genzyme for participation in the ICGG Registry, from Takeda for the GOS Registry, and Pfizer for TALIAS. The Unit also receives research grants from Takeda, Pfizer, Sanofi/Genzyme, and Centogene. M.I. E.S., T.D., D.F., and M.B-C. have no conflict of interest to declare. L.H is the CEO/CTO of Lifeonkey Inc. M.F. is the COO of Lifeonkey Inc. A.Z. receives honoraria from Takeda, Pfizer, and BioEvents and consultancy fees from Takeda, and Prevail therapeutics. S.R.-V. receives grant/research support, honoraria, and travel fee from Takeda, Pfizer, and Sanofi/Genzyme. This study was supported by a grant from Sanofi.

Main screen

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DANGER-ASSOCIATED MOLECULAR PATHWAYS TRIGGERING INFLAMMATION SUGGEST THE BENEFIT OF ADDITIONAL THERAPEUTIC MODALITIES IN GAUCHER DISEASE

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BACKGROUND: Gaucher cells are activated macrophages and are the hallmark for Gaucher disease (GD). Chitotriosidase and CCL18 are selectively secreted from the activated macrophages and are elevated in GD. The
Monocyte-macrophage system, including the peripheral blood mononuclear cells (PBMCs) participate in the activation of the inflammatory pathways. Monocytes sense the “danger” to form the inflammasome that leads to inflammatory cell death(pyroptosis) associated with NLRP3/Caspase-1 pathway. Caspase-1 cleaves pro-IL-1beta, pro-IL-18, which, once cleaved, form oligomers and allow the secretion of mature IL-1beta and IL-18. NLRP3/inflammasome activation has been shown in GD, but consequences cell injury have not been studied. Thus, it is important to elucidate the order of pathogenic events in GD associated with macrophage activation to block the cellular triggers of inflammation in GD.

AIMS: This study investigates the effects of NLRP3/Caspase-1 pathway inhibition on inflammatory markers in GD.

METHODS: PBMC derived from GD and healthy subjects were cultured and active caspase-1 was measured with cell viability. The caspase-1 activity was inhibited with a pancaspase inhibitor, primed with LPS, and activated with nigericin. The levels of IL-1beta, IL-18, and CCL18 were quantified by ELISA.

RESULTS: A significant decrease in L-1beta, and IL-18 was observed with the caspase inhibitor. PBMCs from GD patients, when in the presence of the pancaspase inhibitor, showed decreased caspase-1 activity. The caspase inhibitor increased cell viability in the presence of pancaspase inhibitor.

CONCLUSIONS: Caspase inhibition reduces the macrophage-monocyte activation, which in turn could control disease activity secondary to immune activation and inflammatory response in patients with GD.

DISCLOSURE FOR ALL AUTHORS: No disclosure.

SESSION 5: GBA DEFICIENCY BEYOND GAUCHER DISEASE: PARKINSON’S DISEASE

4 MUTANT LRRK2 IS A MODIFYING GENE OF MUTANT GBA1

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BACKGROUND: Gaucher disease (GD) results from biallelic mutations in the acid beta-glucocerebrosidase (GCase) encoding gene GBA1. Carriers of such mutations have a higher propensity to develop Parkinson’s disease (PD) than the general population. GBA1-related PD is characterized by an earlier age of onset and more severe cognitive symptoms than sporadic PD. PD is a common neurodegenerative disease, and while most cases of PD are sporadic, approximately in 5-10% of PD patients the disease is caused by mutations in several genes, among them GBA1 and LRRK2, both prevalent among the Ashkenazi Jewish population. Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene have been shown to cause autosomal dominant PD, which is milder than the disease associated with a GBA1 mutation. Several recent clinical studies have suggested that carriers of both GBA1 and LRRK2 mutations develop a milder disease compared to the one developed by GBA1 carriers, strongly indicating that there is an interplay between the two genes.

AIMS: In the present study we aimed to test the possible interplay between the GBA1 and LRRK2 genes.

METHODS: We tested the interaction between the GBA1 and the LRRK2 gene products using the fruit fly Drosophila melanogaster as our model.

RESULTS: Our results showed a significant decrease in the Unfolded Protein Response (UPR) levels in double mutant flies, compared to flies carrying the GBA1 mutation alone, alongside a significant reduction in neuroinflammation. A higher rate of dopaminergic cell survival and overall increased locomotor function and longevity of double mutant flies was documented in comparison to all these parameters in GBA1 mutant flies.

CONCLUSIONS: Our results strongly suggest a protective effect of the LRRK2 mutation on mutant GCase-related parkinsonian symptoms in Drosophila flies, recapitulating the known phenotypes in PD patients. Thus, mutant LRRK2 is a modifying gene of mutant GBA1.

DISCLOSURE FOR ALL AUTHORS: None

52 ACID CERAMIDASE PLAYS A KEY ROLE IN THE PATHOGENIC CASCADE LEADING TO NEURODEGENERATION IN GAUCHER AND GBA1-ASSOCIATED PARKINSON’S DISEASE

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BACKGROUND: Brains from patients with neuronopathic GD (nGD) have high levels of GlcCer and GlcSph, a neurotoxic lipid generated by deacylation of GlcCer by the lysosomal enzyme acid ceramidase. Using neurons from induced pluripotent stem cells (iPSCs) derived from patients with nGD, we found that mutant GBA1 deregulated the autophagy/lysosomal pathway (ALP) through hyperactivation of mTOR kinase, a key regulator of lysosomal function. Treatment of nGD neurons with mTOR or acid ceramidase inhibitors prevented mTOR hyperactivation and restored ALP functions, demonstrating that aberrant mTOR activation by glucosphingolipids is involved in pathogenesis, and that GlcSph is the lipid.
species responsible for lysosomal dysfunction in nGD neurons.

AIMS: To examine if this GlcSph/mTOR/pathogenic cascade is involved in abnormal a-synuclein aggregation in dopaminergic (DA) neurons from Parkinson’s disease (PD) patients.

METHODS: To examine this question, we used a stringent isogenic system consisting of iPSC-derived DA neurons from PD patients harboring heterozygous GBA1 mutations (N370S/WT, L444P/WT, and RecNci1/WT), and the corresponding gene-edited WT/WT controls.

RESULTS: The mutant GBA1-DA neurons exhibited mTOR hyperactivation and had elevated levels of pathogenic a-synuclein species compared to isogenic controls. Acid ceramidase inhibition was sufficient to rescue the abnormal mTOR/a-synuclein phenotype of PD-DA neurons, providing compelling evidence that acid ceramidase plays a key role in deregulation of the ALP and the formation of pathogenic a-synuclein species, through aberrant mTOR hyperactivation by GlcSph. This mechanism was confirmed by direct treatment of WT DA neurons with exogenous GlcSph, which recapitulated the abnormal mTOR/a-synuclein phenotype of the mutant PD-DA neurons.

CONCLUSIONS: We conclude that persistent generation of GlcSph by acid ceramidase in GBA1/PD-DA neurons is a key pathogenic event leading to suppression of normal lysosomal functions, including the clearance of pathogenic a-synuclein species. The results suggest that acid ceramidase inhibition may be an effective substrate reduction therapeutic strategy, to prevent or ameliorate neurodegeneration in nGD and GBA1-associated PD.

DISCLOSURE FOR ALL AUTHORS: The authors declare no conflict of interest.

54 PRODROMAL PARKINSONIAN FEATURES IN GBA1 VARIANT CARRIERS

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BACKGROUND: Of the various genetic risk factors to develop Parkinson’s disease (PD), carriers of GBA gene variants (GBA-carriers) are the most common and account for approximately 16% of all patients with PD. It has become evident that several prodromal features may appear 15 to 20 years before the development of the typical motor symptoms. Thus, we initiated a screening study for prodromal features in a large cohort of sequence-proven GBA-carriers with the aim to build a model for identification of a cohort at high risk for PD and to identify those tests with the highest predictive value.

METHODS: GBA-carriers between the ages of 40-75 years were invited to undergo non-invasive tests to assess different domains of PD, including anatomy, cognitive and mental, sleep disorder and motor. In addition, blood was drawn for confirmation of GBA-carrier status and for future analysis.

RESULTS: Herein we report the first 98 subjects (40 males; median age 51 years) enrolled to the study. Twenty-five had at least one relative (1st/2nd degree) with PD. Abnormal REM sleep behavior disorder, UPDRSIII, Epworth Sleepiness Scale (ESS), UPSIT smell test, substantia nigra ultrasound hyper-echogenicity and Montreal Cognitive Assessment (MoCA) were found in 9, 10, 12, 14, 17 and 23 participants, respectively. Significant correlations were found in and between tests from different domains. To define the risk for prodromal PD, we assessed the 10th percentile of each test and detected the outliers, and then calculated the percentage of outliers for each subject. The median [range] outliers were 4.65 [0-50] %, and > 5% outliers were associated with older age and family history of PD.

CONCLUSIONS: We were able to identify 20 subjects with more than 15% outliers who would be eligible for our planned interventional study aimed to delay the onset or even prevent the emergence of motor PD.

DISCLOSURE FOR ALL AUTHORS: The SZMC Gaucher Unit receives support from Sanofi/Genzyme for participation in the ICGG Registry, from Takeda for the GOS Registry, and Pfizer for TALIAS. M.B.-C., T.D., D.A., and E.S. have nothing to disclose. G.Y. receives a consultation fee from Takeda and Abbvie. A.Z. has received honoraria from Pfizer, Takeda (Shire) and BioEvents, and consultancy fees from Prevail Therapeutics and Takeda. S.R.-V. has received research/speaker fees and travel support from Pfizer, Sanofi Genzyme, and Takeda.

64 GBA1 VARIANT AND OTHER LYSOsomAL DISEASE VARIANTS IN CASES OF ATYPICAL PARKINSONISM


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BACKGROUND: Some genes have been implicated as being potentially involved in the pathophysiology of atypical parkinsonism (AP). It is already known that patients with
Gaucher disease (GD) who have pathogenic variants in both alleles of the GBA1 gene and their relatives showed signs of parkinsonism more often than expected. In addition, other genes related to lysosomal diseases to be related to parkinsonism. AP refers to a heterogeneous group of neurodegenerative disorders consists of the following diseases: progressive supranuclear palsy (PSP), multiple system atrophy (MSA), corticobasal degeneration (CBD) and Lewy body dementia (LBD).

AIMS: To verify the existence of variants in genes associated with lysosomal diseases (LD) in patients with AP.

METHODS: Thirteen patients with regular care at the HCPA with the diagnostic criteria for PSP, MSA, CBD and LD were recruited and a control group (patients suspected of having LD). Peripheral blood was collected for DNA extraction. A customized research panel was used to sequence all exons and their boundaries regions of GBA1, SMPD1, LIPA, NPC1, NPC2 and PSAP genes using next-generation sequencing.

RESULTS: Only three patients (pt A, B, C) had sequence variations located in the coding region of target genes: pt A (MSA) - p.Glu365Lys (c.1093G>A) variant located in the GBA1; pt B (PSP) - p.Asn2225Ser variant (c.6676A>G) located in the NPC1; pt C (LBD) - p.Gly492Ser (c.1474G>A) variant in the SMPD1. Sixty-seven control samples were also analyzed, and one patient had the p.Glu365Lys variant located in the GBA1. This patient was diagnosed with Niemann-Pick disease type C.

CONCLUSIONS: Although our results are preliminary, we emphasize that these 3 variants in LD genes could possibly be involved in PA. It is necessary to expand the sample to verify if these genes really have any contribution to AP.

DISCLOSURE FOR ALL AUTHORS: The authors declare no competing interests.

SESSION 6: THERAPIES AND CLINIC: PRESENT & FUTURE

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ELIGLUSTAT IN PATIENTS WITH GAUCHER DISEASE PREVIOUSLY TREATED WITH ENZYME REPLACEMENT THERAPY: REAL-LIFE EXPERIENCE FROM ISRAEL

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BACKGROUND: Three types of enzyme replacement therapies (ERTs) and two substrate reduction therapies (SRTs) are approved in Israel for symptomatic patients with type 1 Gaucher disease (GD1). Eliglustat (Cerdelga™, Sanofi/Genzyme) is the second SRT approved; yet the first SRT approved as first-line therapy to any adult patients with compatible CYP2D6 metabolizer genotype.

AIMS: The aim of this study is to report the safety and efficacy data of the first 42 patients switched from a stable ERT dose to eliglustat from three Israeli centers.

METHODS: All patients with GD1, who have been switched from ERT to eliglustat between 07/2017 and 08/2021, and received at least a single capsule, were included in the analysis for safety. Those who had a follow-up visit at a minimum period of 6 months after switching were included for efficacy analysis.

RESULTS: Most patients switched due to oral preference or sub-optimal response to ERT at a median age (range) of 41 (18-70) years and after a median (range) duration of ERT of 14 years (1-30) (Table). Most of the patients received low-dose ERT. Thirteen patients stopped eliglustat after a median (range) of 3 months (1-18); 11 due to adverse events (AEs) and one due to personal request (Table). All but one drop-outs occurred in one of the centers (SZMC). There were no drug-related serious AEs and no drug-related cardiac events. Most of the AEs were mild and transient, mainly gastrointestinal (dysepsia). Efficacy achievements were reflected by maintaining stability.

CONCLUSIONS: Switching from ERT to eliglustat is safe if choosing the appropriate patients; physicians’ concerns from eliglustat, as reported before, may have affected the relatively high drop-out rate. A stronger reassurance of the patients to tolerate early AEs and perhaps initiation of proton-pump-inhibitors, may reduce early drop-out. A longer follow-up is needed for overall benefit beyond the first 2-years.

DISCLOSURE FOR ALL AUTHORS: The SZMC Gaucher Unit receives support from Sanofi/Genzyme for participation in the ICGG Registry, from Takeda for the GOS Registry, and Pfizer for TALIAS. The Unit also receives research grants from Takeda, Pfizer, Sanofi/Genzyme, and Centogene. M.I., M.B.-C., and T.D. have no conflict of interest to declare. A.Z. receives honoraria from Takeda, Pfizer, and BioEvents and consultancy fees from Takeda, and Prevail therapeutics. T.H. has received honoraria for scientific talks, grants and advice from: Sanofi-Genzyme, Pfizer and Takeda-Shire. J.C. has received honoraria for scientific talks, grants and advice from: Sanofi-Genzyme, Protalix, Pfizer and Takeda-Shire. S.R.-V. receives grant/research support, honoraria, and travel fee from Takeda, Pfizer, and Sanofi/Genzyme. A.Z. receives honoraria from Takeda, Pfizer, and BioEvents and consultancy fees from Takeda, and Prevail therapeutics. N.R-S. has received honoraria for scientific talks, grants and advice from: Sanofi-Genzyme, Pfizer and Takeda-Shire.
PRELIMINARY RESULTS OF TWO YEARS FOLLOW-UP OF TYPE 1 GAUCHER DISEASE PATIENTS TREATED WITH ELIGLUSTAT IN TRAZELGA PROJECT


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BACKGROUND: Eliglustrat (Cerdelga®, Sanofi-Genzyme), is a substrate reduction therapy approved in the European Union in 2015 for Gaucher disease type-1 (GD1) adult patients. TRAZELGA (GEE-ELI-2017-01) project is a prospective follow-up post-authorization study designed as a tool to uniformly study the response to eliglustrat therapy.

AIMS: This study is intending to determine if the drug administration maintains or improve clinical goals and/or plasmatic biomarkers as well as to evaluate eliglustrat therapy response in terms of safety, effectiveness and patient’s satisfaction.

METHODS: Clinical parameters, image bone assessment, and plasmatic biomarkers were analyzed for two years on follow-up: chitotriosidase [ChT] activity was evaluated by fluorimetry, glucosylphosphinosine [GluSph] concentrations by LC-MSMS and CCL18/PARC, C5a, C3, YKL40, Cathepsin S, Hecpidin and Lipocalin concentrations by immunoquantification assay. A quality of life study (SF-36), disease severity score index evaluation (GD-DS3) and compilation of side effects are included. Non-parametric statistical analysis was performed and p<0.05 was considered as statistically significant.

RESULTS: Twenty-Nine Spanish GD1 patients were included, 16 were males and 13 female. The median age was 42.0 (30.00-55.50) years. All patients were treated before starting eliglustrat: 26 with enzyme replacement therapy, 2 with substrate reduction therapy and 1 with a combined therapy. The median age of treatment before eliglustrat was 17.0 (10.00-21.50). A statistically significant decrease on GluSph and CCL18/PARC (p=0.0393 and p=0.0019, respectively) was observed meanwhile ChT was decrease on GluSph and CCL18/PARC (p=0.0393 and p=0.0019, respectively) was observed meanwhile ChT was.

CONCLUSIONS: Eliglustrat maintains or improve biochemical biomarkers after two years treatment.

DISCLOSURE FOR ALL AUTHORS: TRAZELGA (GZ-2017-11713) received a grant from Sanofi for its realization.

23 PHARMACOKINETICS AND BIOMARKER RESPONSES IN PATIENTS WITH GAUCHER DISEASE TYPE 3 OR GBA-ASSOCIATED PARKINSON’S DISEASE TREATED WITH VENGLUSTAT

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BACKGROUND: Venlustomat is an oral, brain-penetrant, glucosylceramide synthase inhibitor under investigation for treatment of neurologic features of Gaucher disease type 3 (GD3, an inherited metabolic disorder arising from GBA mutation) and formerly under investigation for Parkinson’s
disease with GBA mutations (GBA-PD). Mutations in GBA, which encodes acid β-glucosidase, result in reduced enzymatic activity and accumulation of glucosylceramide (GL-1) and glucosylsphingosine (Lyso-GL-1). In healthy subjects, venglustat was well tolerated and reduced plasma GL-1 levels within 2 weeks in a dose-dependent manner.

**AIMS:** Venglustat pharmacokinetics and biomarkers (GL-1 and Lyso-GL-1) from Phase 2 studies of GD3 and GBA-PD are reported.

**METHODS:** In the LEAP trial (NCT02843035) 15 mg venglustat once-daily combined with imiglucerase was evaluated in GD3 patients (N=11). In the randomized, placebo-controlled MOVES-PD Part 1 dose-escalation trial (N=29), Part 2 double-blind trial evaluating 15 mg once daily (N=221).

**RESULTS:** Mean (SD) CSF concentrations following 15 mg oral venglustat were 6.63 (2.42) ng/mL in LEAP at Week 4, 8.02 (2.40) ng/mL in MOVES-PD Part 1 at Week 4, and 6.28 (2.26) ng/mL in MOVES-PD Part 2 at Week 52. Venglustat plasma concentrations indicated steady state was achieved on or before 4 weeks. Median (IQR) GL-1 in CSF decreased 81% (77-83) in LEAP at Week 52, 73% (70-79) in MOVES-PD Part 1 at Week 4, and 75% (58-83) in MOVES-PD Part 2 at Week 52. Median (IQR) Lyso-GL-1 in CSF decreased 70% (46-76) in LEAP at Week 52. Lyso-GL-1 was undetectable in CSF at all time-points in MOVES-PD. Reductions in plasma biomarkers were similar to CSF biomarkers. Both trials reported favorable safety profiles.

**CONCLUSIONS:** Venglustat crossed the blood-brain barrier and steady state was achieved on or before 4 weeks of treatment. Venglustat treatment resulted in marked reductions in GL-1 and Lyso-GL-1 levels in CSF and plasma.

**DISCLOSURE FOR ALL AUTHORS:** JP, JS, LK, AJ, PM are employees and/or stockholders at Sanofi.

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**ELISAFE: BASELINE CHARACTERISTICS FROM AN OBSERVATIONAL STUDY TO EVALUATE REAL-WORLD SAFETY OF ELIGLUSTAT IN PATIENTS WITH GAUCHER DISEASE**

I. Batsu ¹, B. Accomando ², S. Gaemers ³, D. Sekulić ¹

**BACKGROUND:** Eliglustat, a glucosylceramide synthase inhibitor, is approved as first-line oral therapy for adults with Gaucher disease (GD) type 1 who are extensive, intermediate, or poor CYP2D6 metabolizers. ELISAFE is a real-world, observational, post-authorization study of the long-term safety of eliglustat in participants with GD, a safety sub-registry of the International Collaborative Gaucher Group (ICGG) Gaucher Registry (NCT00358943).

**AIMS:** The primary objectives of ELISAFE are to evaluate eliglustat long-term safety and describe utilization of eliglustat in real-world clinical practice. This report focuses on ELISAFE baseline characteristics at enrolment closure (30 December 2020).

**METHODS:** Adults ≥18 years old, with GD and receiving treatment with eliglustat or imiglucerase, who enrolled in the ICGG Gaucher Registry were eligible for ELISAFE. Planned enrolment was ≥100 patients receiving eliglustat, with an eliglustat:imiglucerase treatment ratio of 2:1.

**RESULTS:** Overall, 165 patients were enrolled in ELISAFE (eliglustat, n=110; imiglucerase, n=55). Mean ± standard deviation (SD) baseline age was 43±14 years, 49% were women, and 97% were diagnosed with GD type 1; the eliglustat and imiglucerase groups were similar for these characteristics. Mean±SD age at GD onset was 23±15 years (eliglustat 25±15 years; imiglucerase 21±16 years). Mean±SD time since GD diagnosis was 20±12 years (eliglustat 19±12 years; imiglucerase 22±12 years) and mean±SD time on GD treatment before enrolment was 14±9 years (eliglustat 13±9 years; imiglucerase 16±8 years); 97% of participants were experienced to any GD treatment. Among eliglustat-treated patients with CYP2D6 phenotype data available, 69% were extensive, 20% were intermediate, and 6% were poor metabolizers. At baseline, 88% of eliglustat patients were receiving 84mg twice daily; 93% of imiglucerase patients were receiving one infusion every 2 weeks (median dose: 35 U/kg).

**CONCLUSIONS:** Baseline data suggest that the enrolment goals for ELISAFE have been achieved.

**DISCLOSURE FOR ALL AUTHORS:** IB, BA, SG, and DS: Employees of Sanofi Genzyme and may hold shares and/or stock options (<5%) in the company.

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**47 VENGLUSTAT, A NOVEL, INVESTIGATIONAL, BRAIN-PENETRANT GLUCOSYLCERAMIDE SYNTHASE INHIBITOR, FOR GAUCHER DISEASE TYPE 3: PHASE 3 LEAP2MONO TRIAL DESIGN**

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**BACKGROUND:** Venglustat is a novel, small-molecule, brain-penetrant glucosylceramide synthase inhibitor under investigation as a disease-modifying therapy for Gaucher disease type 3 (GD3). Imiglucerase enzyme replacement therapy (ERT) ameliorates hematologic, visceral, and bone manifestations of GD3; however, there is currently no treatment for the neurologic manifestations. After one year of venglustat+imiglucerase in the Phase 2 LEAP trial (NCT02843035/Sanofi), venglustat concentration was maintained in CSF, median glucosylceramide and glucosylsphingosine in CSF decreased markedly, and potential to ameliorate neurological manifestations was demonstrated.
AIMS: Describe the design of the LEAP2MONO study of venglustat monotherapy for GD3.

METHODS: LEAP2MONO is a Phase 3, randomized, double-blind, trial evaluating efficacy and safety of venglustat vs imiglucerase in adults and children ≥12 years with GD3 who have reached therapeutic goals with ERT. Patients will be randomized to receive either once-daily oral venglustat with biweekly placebo infusions or biweekly imiglucerase infusions with once-daily oral placebo for 52 weeks (primary analysis period). Venglustat patients who meet prespecified criteria for decline in GD status can receive ERT rescue therapy. After Week 52, all patients will receive venglustat for 52 weeks (extended treatment period). Safety follow-up will continue through 30-37 days after last treatment. Enrollment is planned for 40 participants, including 10 pediatric participants. Primary endpoints are change from baseline to Week 52 on the Scale for Assessment and Rating of Ataxia modified total score and the Repeatable Battery for the Assessment of Neuropsychological Status total scale index score. Secondary endpoints include safety, tolerability, and changes from baseline in spleen volume, liver volume, hemoglobin, and platelets. Exploratory endpoints include changes from baseline in glucosylceramide and glucosylsphingosine in plasma and CSF, venglustat pharmacokinetics, and patient-reported outcomes. This study will evaluate whether the effects of venglustat monotherapy translate to clinical benefit on neurological manifestations with maintenance of systemic disease stability in GD3 patients.

RESULTS: Nothing

CONCLUSIONS: Nothing

DISCLOSURE FOR ALL AUTHORS: IB, WH, PM, DC are employees and/or stockholders at Sanofi.

15 BRAIN-PENETRANT STRUCTURALLY TARGETED ALLOSTERIC REGULATORS FOR GLUCOCEREBROSIDASE (GCASE) SHOW PROMISING PHARMACOLOGICAL ACTIVITY IN L444P MODELS OF GAUCHER DISEASE

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BACKGROUND: Gaucher disease (GD) is a lysosomal storage disorder characterized by deficiency of the GCase activity due to a mutation in the GBA1 gene. Decreased GCase activity is associated with accumulation of its toxic substrate glucosylceramide in several organs, and in some cases, also a critical neural dysfunction occurs. Homozygous GD patients harbouring the L444P*GBA1 mutation are at a high risk of developing neuronal impairment.

Since current drugs for GD are non-brain penetrant, there is an unmet medical need that stimulates the development of therapies that can improve the neurological phenotype of GD patients. Gain Therapeutics is developing pharmacological chaperones, small molecules able to cross the blood-brain barrier that prevent GCase protein misfolding, followed by the restoration of the enzyme function.

AIMS: Demonstrate the engagement of our structurally targeted allosteric regulator (STAR) GD candidate in increasing L444P*GBA1 enzyme activity in the lysosomes in both GD patients’ cells and in an animal model harboring the mutation.

METHODS: Gain Therapeutics has applied its innovative proprietary drug discovery platform, Site-directed Enzyme Enhancement Therapy (SEE-Tx™), to the development of small-molecule STAR that stabilize misfolded GCase and increase its activity.

RESULTS: We report in vitro and in vivo increase of GCase activity upon treatment with our STAR GD candidate, in fibroblasts derived from GD patients, and in plasma of homozygous L444P/L444P mice after 14 days of oral treatment.

CONCLUSIONS: We report that our orally bioavailable STAR increases L444P*GBA1 enzymatic function with potential as a therapy to mitigate neurological effects in GD alone or in combination with other existent therapies. Thus, STARs represent a novel pharmacological tool for the treatment of GD, warranting development towards the clinic.

DISCLOSURE FOR ALL AUTHORS: No disclosure to submit.

18 THE SURVIVAL AS RELATES TO THE CLINICAL SPECTRUM, MOLECULAR VARIANTS AND CHAPERONE RESPONSE IN ACUTE NEURONOPATHIC GAUCHER DISEASE

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BACKGROUND: The definition of acute neuronopathic GD(GD2) is the onset of neurological disease by 6 months and death before age 4 without life support. GD2 is associated with supranuclear gaze palsy, stridor and apnea necessitating tracheostomy, abnormal swallowing requiring G-tube placement, progressive myoclonus and failure to achieve independent gait. GD2 starts in utero with a pre/perinatal presentation.

AIMS: To present the clinical spectrum, GBA variants, GCase activity and in vitro chaperone response in GD2.

METHODS: Twelve infants with a suspected diagnosis of GD2 were evaluated. The pharmacologic chaperone Ambroxol was tested in PBMCs and/or fibrolasts at 10 microM concentration, and response was compared to overall survival, and other morbidities.
NEW GLYCOLITE PLAYERS IN GAUCHER DISEASE

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BACKGROUND: In recent years we started to realize that glucocerebrosidase (GCase) may have more substrates than solely glucosylceramide (GlcCer). This may have implications for our view on Gaucher disease and therapy strategies.

Joint research at the departments of Medical Biochemistry and Bio-organic Synthesis at the Leiden Institute of Chemistry has allowed us to identify several hitherto unknown glycosylated metabolites that seem to be synthesized by the enzyme Gba2 and degraded by GCase. Observed accumulation of some of these ‘glycolites’ in GD materials suggests that they may be more than products of ‘exotic laboratory biochemistry’. An overview of the latest data on the fascinating glycolites will be presented. Which glycolites are now identified and how are further ones being discovered. Finally, their potential physiological relevance will be discussed.

N-ACETYLCYSTEINE AMIDE (NACA), A NOVEL

RESULTS: The median age at diagnosis was 7 months. Three were identified through Newborn Screening. Three distinct phenotypes were early onset, associated with skin manifestations and worst prognosis; classical and nonclassical forms. Common presentations were hepatosplenomegaly and thrombocytopenia. Other findings included stridor(7/11), abnormal ABR(11/11), and swallowing(9/12), opisthotonus 3/11), seizures(4/11) abnormal liver enzymes, cholestatic jaundice (2/11). Facial dysmorphism was identified with classical GD2. The most common GBA variant was L444P(8/12). Ambroxol response was negative if GCase increased 20% or less (3/9), associated with death by 24 months or ventilatory support, and positive if GCase increased 100% or more(2/9), a survival without tracheostomy beyond age 5 or mild delay and walking by 15 months. For moderate response, disease progression usually correlated with the neurologic status at diagnosis.

CONCLUSIONS: The diagnosis of GD2 is problematic in early life. This case series illustrate the distinct GD2 clinical forms. L444P combined with a complex/recombinant allele results in classical GD2. Therapeutic advances such as gene therapy, makes the identification curial before irreversible symptoms. Chaperones could not only increase survival and modify the course but may aid in identification of attenuated forms.

DISCLOSURE FOR ALL AUTHORS: Nothing to disclose.
THERAPEUTIC OPTION TO POTENTIALLY ADDRESS NEUROLOGICAL MANIFESTATIONS OF GAUCHER DISEASE

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BACKGROUND: In Gaucher Disease (GD), the misfolded glucocerebrosidase (GCase) enzyme and the resulting glycolysogolipid accumulation can trigger oxidative stress leading to various neurological manifestations. In addition to neuronopathic GD types 2 and 3, recent studies have shown that patients with non-neuronopathic type 1 GD, have altered neurochemical profiles and GBA1 mutations are associated with Parkinsonism. However, none of the current treatments address the neurological aspects of GD since these do not cross the blood-brain barrier (BBB). N-acetylcysteine amide (NACA), a small molecule with antioxidant potential, high bioavailability, and greater ability to cross the BBB, can be a novel therapeutic option for GD. NACA is currently in Phase II clinical trial to address the oxidative stress underlying retinal disease associated with Usher syndrome. NACA is the prodrug of N-acetylcysteine (NAC), an FDA-approved antioxidant. NAC acts as a chaperone and can improve GCase activity. In this study, we evaluated the efficacy and safety of NACA using in vitro GD models.

AIMS: Our objective is to examine NACA is safe in GD and can improve cellular function as a pharmacological chaperone and antioxidant.

METHODS: Three GD skin fibroblast cell lines were used. Cells were incubated with NACA at a concentration of 0-1 mM for 24-72 h. Cell viability was measured using CyQuantTM NF Cell proliferation Assay (Invitrogen, MA). Mitochondrial superoxide level was measured using MitoSox Red fluorescence probe (Invitrogen, MA). Further, 0.5 mM NACA significantly decreased up to 53% mitochondrial superoxide levels in a mutation-dependent manner.

RESULTS: GD fibroblasts tolerated increasing the concentration of NACA up to 1mM in a time-dependent manner. Further, 0.5 mM NACA significantly decreased up to 53% mitochondrial superoxide levels in a mutation-dependent manner.

CONCLUSIONS: NACA was observed to be safe and effective in decreasing mitochondrial oxidative stress in vitro. Further studies will confirm its antioxidant capacity and enzyme activity in GD fibroblasts.

DISCLOSURE FOR ALL AUTHORS: JR and MRT have no disclosure. RVK has received grants from Sanofi, Pfizer Inc., and the National Institutes of Health.

75 NOVEL AAV9-BASED GBA1 GENE REPLACEMENT THERAPY FOR GAUCHER DISEASE AND PARKINSON’S DISEASE WITH PATHOGENIC GBA1 MUTATIONS

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BACKGROUND: Gaucher disease (GD) is a lysosomal storage disease manifesting as a clinical spectrum, from symptoms restricted to the periphery (type 1, GD1) to neurologic impairment (severe in type 2, GD2; variable in type 3, GD3). The root cause of GD is deficiency in the glucocerebrosidase (GCase) enzyme due to bi-allelic pathogenic mutations in the GBA1 gene. Mutations in GBA1 (monoallelic and bi-allelic) are also the most common known genetic risk factor for the development of Parkinson’s disease (PD). Mutant GCase results in diminished enzymatic activity, and the level of activity generally correlates with disease severity. LY3884961 is an investigational gene therapy that delivers a healthy human copy of the GBA1 gene using recombinant AAV9 and is being investigated as a single-dose gene therapy for GD and PD patients caused by GBA1 mutations.

AIMS: Based on supportive data from preclinical studies, LY3884961 is being evaluated in multiple clinical indications related to deficient GCase activity.

METHODS: Open-label investigation of single-dose LY3884961 is ongoing in Phase 1/2, safety, dose-finding, tolerability, and exploratory efficacy studies.

RESULTS: Study enrollment is ongoing at sites globally, and safety, tolerability, and exploratory efficacy endpoints are being collected.

CONCLUSIONS: One-time administration of LY3884961, an AAV9-GBA1 investigational gene therapy, provides an opportunity to assess whether a single-dose treatment can provide meaningful clinical benefit in the spectrum of diseases caused by GBA1 mutations. Phase 1/2 clinical studies are ongoing.

DISCLOSURE FOR ALL AUTHORS: (1) I am submitting this abstract with the intent of attending the IWGGD 2022 Scientific meeting and presenting in person. (2) I confirm each co-author has been informed of this abstract submission and has agreed to all information as it was submitted. (3) I confirm that disclosures of all co-authors were declared.

POSTERS

BIOMARKERS

35 CHITOTRIOSIDASE ACTIVITY CORRELATION IN DRIED BLOOD SPOTS AND PLASMA IN GAUCHER PATIENTS.

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BACKGROUND: Chitotriosidase (ChT) enzyme is significantly higher in Gaucher disease (GD), some LSDs and others no lysosomal disease. ChT activity had been recognized as a biomarker to diagnosis and treatment monitoring for therapy in GD. The analysis by ChT activity can be determined mainly in plasma, however, dried-blood spot (DBS) samples had been used for the same aid. Nevertheless, in Western Europe population ChT deficiency and carrier occur in 6% and 30% due to a 24 bp duplication in the CHIT1 gene (MIM*600031).

AIMS: Evaluate the ChT activity correlation between DBS and plasma samples in GD patients.

METHODS: Biobank of Health System in Aragón provided 108 GD patients samples with confirmed enzymatic and genetic diagnosis. ChT activity was measured by duplicate in plasma and DBS through 4-methylumbelliferyl-β-D-N,N',N''triacetylchitotrioside as described previously Hollak et al., and Chamoles et al., respectively. CHIT1 genotpye for the variant NM_001256125.2:c.1049_1072dup24 was analyzed by RFLP in all samples. Comparison between both plasma and DBS levels were made by non-parametric tests (Spearman’s correlation) and linear regression models.

RESULTS: Only 3.7% of samples was excluded due to null activity for ChT. Median (Q1-Q3) ChT activity in DBS values were 0.82 (0.55-1.62) nmol/punch/h meanwhile plasma values were 442.43 (217.32-948.69) nmol/mL/h. The coefficient of correlation (r) was 0.84 but the coefficient of determination (r²) was moderate (0.65) with a linear regression y= 924.78x - 262.16.

CONCLUSIONS: There is a correlation between both samples, validating the utility is DBS as a matrix for ChT activity determination. Despite they are rising in unison, the variation between variants cannot be explain just by each other, perhaps if DBS data was corrected by other factors as the leukocytes count or the hematocrit, the coefficient of determination will be increased.

DISCLOSURE FOR ALL AUTHORS: Nothing to disclosure.

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LYSO-GB1 LEVELS IN A COHORT OF GAUCHER DISEASE PATIENTS

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BACKGROUND: Glucosylsphingosine (lyso-Gb1) is a deacylated form of glucosylceramide that has proven to be a specific biomarker for the follow-up of GD patients.

AIMS: We herein report the lyso-Gb1 levels of a cohort of GD patients to better understand the range of this biomarker in different clinical contexts.

METHODS: This is a transversal study. GD patients are seen at the GD Reference Center at the Hospital de Clínicas de Porto Alegre (HCPA), Brazil. We included 29 patients: 22 treated adult type 1 GD patients (GD1, female = 13, mean age of 45 ± 15 years, 12 on enzyme replacement therapy (ERT) and 1 in SubstrateReductionTherapy), one treated 11-year-old GD1, three treated adult patients with type 3 GD (GD3, male=2, all on ERT), and three untreated GD patients (one GD1, one type 2 (GD2) and one GD3). We also compared it with levels of two different control groups (general newborns, and other LSDs). Lyso-Gb1 was quantified by liquid chromatography-tandem mass spectrometry,results were expressed as nmol/L.

RESULTS: The average of lyso-Gb1 for the treated GD1 was 147 (range:15-793) and for the 11-year-old patient was 394. The average value for the treated GD3 was 743 (range 644-837). The values for the untreated GD patients were 395, 542 and 3447.4 respectively for GD1, GD2, and GD3. The average of lyso-Gb1 for newborns was 6 (range: 3-12) and for other LSDs was 7 (range: 5-11). The average treatment time before the measurement was 14 ± 5 years.
CONCLUSIONS: This is the first experience of our center using lyso-Gb1 as a biomarker. Higher levels of lyso-Gb1 were seen in GD2 and both treated and untreated GD3 when compared to GD1. This study can help to better understand the cut-off lyso-Gb1 values for different clinical contexts.

DISCLOSURE FOR ALL AUTHORS: Conflict of Interest Statement. The authors have no ethical conflicts to disclose.

GBA DEFICIENCY: MALIGNANCIES AND PARKINSON’S DISEASE

41 MUTANT ZEBRAFISH AS RESEARCH MODEL TO STUDY CONSEQUENCES OF GCase DEFICIENCY

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BACKGROUND: In Gaucher disease (GD), deficiency of glucocerebrosidase GBA1 causes lysosomal accumulation of glucosylceramide (GlcCer) which is partly converted by acid ceramidase (ASAH1) to glucosylsphingosine (GlcSph). To study the role of excessive GlcSph formation during glucocerebrosidase deficiency we studied zebrafish that have two orthologues of acid ceramidase, Asah1a and Asah1b. Only the latter is involved in formation of GlcSph in glucocerebrosidase-deficient zebrafish.

AIMS: Comparison of Gaucher zebrafish with excessive GlcSph (gba1-/- fish) and without GlcSph (gba1-/-:asah1b-/- fish) in order to identify consequences of chronic high levels of GlcSph.

METHODS: We compared various fish at different ages with regard to lipid composition, presence of lipid-laden storage cells, inflammation and motor behavior.

RESULTS: Prevention of excessive GlcSph in gba1-/-:asah1b-/- fish did not restrict storage cells or GlcCer accumulation. No significant increases in these parameters were detected in gba1-/-:asah1b-/- fish. No significant effect of the prohibition of GlcSph formation was noted on neuroinflammation as determined by gene expression analysis. However, GD fish lacking excessive GlcSph show an ameliorated course of disease reflected by significantly increased lifespan, delayed locomotor abnormality, and delayed development of an abnormal curved back posture. Loss of putative dopaminergic neurons expressing tyrosine hydroxylase 1 (th1) mRNA is slowed down in GD fish lacking excessive GlcSph.

CONCLUSIONS: In the zebrafish GD model, excess GlcSph has little impact on (neuro)inflammation or the presence of GlcCer-laden macrophages, but rather seems harmful to th1-positive dopaminergic neurons. It should be kept in mind that the studied mutant zebrafish offer not a straightforward model for human GD-PD. Formation of deacylated sphingolipid bases is observed with other lysosomal sphingolipidoses. It is presently studied whether asah1b-/-fish are helpful to shed light on consequences of lyso lipids in other LSDs.

DISCLOSURE FOR ALL AUTHORS: No disclosure to submit.

GBA GENE AND PROTEIN

33 A VALIDATE NOVEL ENZYMATIC DIAGNOSIS METHOD OF LYosomal DISEASES IN DRIED BLOOD SPOTS

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BACKGROUND: Lysosomal storage diseases (LSD) are usually caused by enzymatic activity deficiencies. The gold standard for the diagnosis is the quantification of their enzyme activity in blood samples, extracts of leukocytes or cultured fibroblasts, mostly using chromogenic substrates.

AIMS: To present a novel enzymatic method in dried blood spots (DBS) for rapid screening of LSD.

METHODS: Based on the fluorophore Cy3 linked to R-β-glucose (activity marker for Gaucher disease), 2-hexadecanoylamino-4-R-phosphorylcholine (activity marker for ASMD), and R-α-globotriaosylceramide (activity marker for Fabry disease), allow quantifying activities associated to those diseases in blood samples. Resuspended blood from DBS was used for the assays to establish the associations between the enzymatic activity related to these substrates and susceptibility to the diseases in individuals previously diagnosed.

RESULTS: After blind validation 100% concordance was seen with 78 previously characterized LSD samples, including Acid Sphingomyelinase Deficiency, Gaucher and Fabry diseases, the technique was further implemented to test 1922 additional DBS from consecutive newborns. The preliminary results herein presented suggest that it is feasible to incorporate the new substrates for the enzymatic diagnosis of the herein studied, and potentially other LSD. Compared to fluorimetric methods, based on umbelliferone derivatives, this method allows significantly reducing the assay time (completed in 1 h), and increasing...
the detection limit (from 0.2 μg mL⁻¹ to less than 0.001 μg mL⁻¹) so smaller amount of substrate and smaller amount of blood sample is needed (by 10-50 fold less). Blood samples from healthy individuals had a median activity value of 5.53 (3.43, interquartile range [IQR]), 5.35 (3.51, IQR), and 5.35 (3.51, IQR) μmol/L/min, respectively. In affected patients the values below 0.3 μmol/L/min, on average.

CONCLUSIONS: This enzymatic method is available that can identify enzyme deficiencies associated with lysosomal diseases at low cost and with minimal sample quantity and time.

DISCLOSURE FOR ALL AUTHORS: I am submitting this abstract with the intent of attending the IWGGD 2022 Scientific meeting and presenting. I confirm each co-author has been informed of this abstract submission and has agreed to all information as it was submitted.

INCIDENCE OF GD

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A SYSTEMATIC LITERATURE REVIEW OF THE GLOBAL INCIDENCE AND PREVALENCE OF GAUCHER DISEASE

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BACKGROUND: Epidemiological estimates for Gaucher disease (GD) can be reflective of localized or health-seeking study populations, generating heterogenous data. A broader overview is needed to examine the generalizability of these estimates to different geographic regions.

AIMS: To provide a qualitative synthesis of global GD incidence and prevalence estimates, overall and by disease type, published in the last 10 years.

METHODS: Comprehensive literature search was conducted across multiple databases from 1 January 2011–30 September 2020, and congress proceedings to 6 May 2021. Studies reporting incidence/prevalence estimates for patients with GD types 1–3, or data from which these could be derived, were evaluated.

RESULTS: In total, 490 publications were identified, of which 31 were selected for analysis: 20 cohort studies (15 prospective, 5 retrospective), 6 cross-sectional studies and 5 online reports. Most studies originated from Europe (n=11) or North America (n=11). One study was multi-regional. Across all GD types, incidence estimates ranged from 0.45–25.0/100,000 live births (16 studies), and were lower in the Asia-Pacific than other regions (Table). Prevalence estimates (any GD type) ranged from 0.02–139.0/100,000 inhabitants from 17 studies; estimates were higher in North America than other regions (Table). Highest prevalence was from a North-American Ashkenazi-Jewish population (139/100,000 population). GD3 incidence was reported in Asia-Pacific only (1.36/100,000 live births), and GD2 and GD3 prevalence were reported in Europe only (0.02-0.08/100,000 population) (Table). Generalizability of estimates were assessed as adequate or intermediate for all regions with data.

CONCLUSIONS: GD incidence and prevalence estimates for the last 10 years varied considerably between regions, and are poorly documented in regions outside Europe and North America. Limited information is available for GD2 and GD3. Study and writing funded by Takeda.

DISCLOSURE FOR ALL AUTHORS: Genaro Castillon, Employee of YolaRX Consultants Inc, which received funding from Takeda. Yola Moride, President of YolaRX Consultants Inc, which received funding from Takeda. Shun-Chiao Chang, Employee and stockholder of Takeda Pharmaceuticals Company Ltd.

NEURONOPATHIC GD

61
LONG TERM FOLLOW UP OF LYMPHADENOPATHY IN EGYPTIAN GAUCHER DISEASE CHILDREN AND ADOLESCENTS

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BACKGROUND: Lymphadenopathy is an under-recognized manifestation of Gaucher disease(GD) with 46 cases reported in the literature. Lymph nodes involved and/or described are usually mesenteric and/or mediastinal and can be associated with lung disease. Lymphadenopathy can be progressive with life threatening complications. Most studies are case series and few described the characteristics and the course of these lymph nodes in GD patients receiving enzyme replacement therapy (ERT)over a long duration.

AIMS: We conducted this study to assess prevalence and characteristics of lymphadenopathy in our GD cohort and its response to ERT.

METHODS: All children diagnosed with Gaucher disease having significant abdominal symptoms, severe visceral or lung disease were evaluated by CT abdomen and HRCT to assess for lymphadenopathy and lung disease respectively.

RESULTS: Twenty-Five (4 GD1, 21 GD3/130, 19.2 % subjects; 13 males, 12 females) with a mean age of 11 years from 20 families; 19 L444P/L444P, 1 R359 Q/3559Q, 5 unknown were enrolled. Four children were diagnosed on initial presentation and 21 while receiving ERT. Only 5 (20%) patients had significant abdominal pain and/or change in bowel habits. All patients had mesenteric lymph nodes,9(45%) had both mediastinal and mesenteric lymph nodes, and 2 had generalized lymphadenopathy. In 15 (60%) GD3 patients severe neurological involvement was noted whilst 18(72%) GD patients have lung disease of variable severity. Nine (7 children and 2 adolescents) GD
patients passed away mostly due to SUDEP (sudden unexplained death in epilepsy), a phenomenon reported in our Egyptian cohort. The course of lymphadenopathy was either stationary or very slowly regressive and none showed progression.

CONCLUSIONS: Lymphadenopathy in GD Egyptian patients is not rare and all children diagnosed with GD should be screened for lymphadenopathy as it can be considered a marker of disease severity.

DISCLOSURE FOR ALL AUTHORS: None

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R463C, THE CHAMELEON VARIANT: PHENOTYPES ASSOCIATED WITH A MODERATELY SEVERE GBA1 MUTATION

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BACKGROUND: For a ‘simple’ mendelian disorder, Gaucher disease (GD) is marked by vast clinical variation and limitations in genotype/phenotype correlation. This heterogeneity has serious implications for patients, medical providers and researchers, and complicates patient education, follow-up recommendations and determining prognosis. The GBA1 variant R463C (p.Arg502Cys) is a prime example, as it is found among patients with the full spectrum of GD, including non-neuronopathic (GD1), chronic (GD3) and acute neuronopathic (GD2) disease.

AIMS: To further characterize variant R463C as a resource for the GD community.

METHODS: A literature search identified reports of the variant in 13 countries, including Great Britain, Israel, Greece, Turkey, Portugal, India, and the USA. Reports of these 53 cases were combined with an in-depth analysis of 25 current and historical patients seen at the NIH and followed over 3-25 years.

RESULTS: Combined, 22 different genotypes with variant R463C were reported. Of the 25 NIH patients examined, 11 were classified as GD3 and 14 as GD1. Onset of symptoms generally occurred in early childhood (mean 4.3 years) regardless of classification. In most cases the phenotype corresponded to what would be expected from the second mutation. For some historical cases we had inadequate phenotypic documentation and/or follow-up.

CONCLUSIONS: R463C is a moderately severe variant, as homozygotes tend to have neuronopathic disease, albeit relatively mild. The clinical phenotype is often dictated by the second variant. Careful and repeated assessments are needed as the neuronopathic involvement can be subtle, with mild saccadic involvement and electroencephalogram abnormalities. Continued evaluations are necessary, as patients may develop neuronopathic features later in life, and historical cases or young children require reassessment for accurate diagnosis and variant reporting.

NEW DEVELOPMENTS AND YOUNG RESEARCHERS

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DELINEATING THE DETERMINANTS OF OSTEONECROSIS IN GAUCHER DISEASE TYPE 1 IN THE ERA OF THERAPIES

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BACKGROUND: A dramatic clinical impact of enzyme replacement therapy (ERT) has been striking reduction of incidence of Avascular Osteonecrosis (AVN) and joint deformities requiring surgical intervention.

AIMS: Nevertheless, occasional anecdotal episodes of AVN have been reported on ERT. We conducted a retrospective study spanning 20 years in a large tertiary referral center to assess the frequency of AVN on treatment and its determinants.

METHODS: The study was conducted in 156 GD1 patients followed longitudinally between 2001 and 2020, 78F and 78M at a tertiary clinic. The collected data included age and gender, type of treatment (imiglucerase, velaglucerase, and eliglustat), history of AVN pre-treatment, genotype ascertained by full GBA1 sequencing, MRI volumetrics for liver and spleen, CBC, and biomarkers including glucosylsphingosine (GlcSph). We applied mixed logistics model (SPSS 28) to delineate the independent correlates of AVN on treatment.

RESULTS: The patients received 1642 years of treatment. During 20 years, there were 15 episodes of AVN in 13 patients, with two episodes, each occurring in two patients. One patient on velaglucerase developed pan-reactive neutralizing antibodies to all ERTs. Heteroallelic N370S GD1 patients were 9.46 times (95% CI, 1.35-66.2) more likely than N370S homozygous patients to develop osteonecrosis during treatment. History of AVN prior to treatment initiation increased 6.09 fold risk of AVN on treatment (95% CI, 1.66-22.2). The risk of AVN among patients who had received velaglucerase-ERT was 3.99 times higher compared to patients receiving imiglucerase-ERT (95% CI, 1.37-11.59). No patient receiving eliglustat SRT suffered AVN. There was a significant correlation between GlcSph levels and AVN (95% CI, 1.001-1.01).
CONCLUSIONS: There is a low but significant risk of AVN in GD1 in the era of ERT/SRT. We found increased risk of AVN related to genotype,history of AVN prior to treatment initiation, GlcSph levels, and type of ERT.

DISCLOSURE FOR ALL AUTHORS: Dr. Mohsen Basiri is Sanofi LSD Training Fellow. Dr. Pramod Mistry has received research grant support and travel support from Sanofi Genzyme.

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A PROTOCOL FOR A MULTIDIMENSIONAL ASSESSMENT IN GAUCHER DISEASE

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BACKGROUND: Gaucher disease (GD) is a genetic lysosomal disease characterized by a deficiency of the lysosomal enzyme β-glucocerebrosidase, resulting in an accumulation of glucosylceramide in macrophages in the body. Patients with GD type 1 usually present different processes of disability and pain throughout their lives that have a negative impact on their daily life. Through the assessment of factors of a different nature (psychosocial, biological, environmental...) we can get to know to develop tools to address them.

AIMS: To analyze Gaucher patients with type 1, in order to bring in the maximum and most detailed information possible after to do a multidimensional assessment in these patients.

METHODS: A protocol for conducting a cross-sectional, descriptive, and observational study is presented, in which, the level of physical activity, fatigue, pain, quality of life, depression, stress and level of anxiety will be measurement with IPAQ, take into account steps/day, FACIT-Fatigue, VAS, SF-36 and PSS respectively. Patients are contacting via phone and email. In this email, the therapist will explain the way to fill in the scales and questionnaires. This process of carrying out surveys will be carried out again at 3 and 6 months. An analysis of the data obtained from the different questionnaires will be carried out and the results obtained will be interpreted.

RESULTS: The expected results are the achievement of detailed information on the pain and disability processes suffered by type I GD patients.

CONCLUSIONS: There are few studies conducted on the treatment of GD with physical activity. Furthermore, nor are there many studies dedicated to knowing the musculoskeletal disorder that this disease generates, and technology has a huge potential in order to facilitate the population’s access to information and encourage changes to healthier lifestyles and favor the autonomy of people during the processes of loss of health.

DISCLOSURE FOR ALL AUTHORS: I am submitting this abstract with the intent of attending the IWGGD 2022 Scientific meeting and presenting. I confirm each co-author has been informed of this abstract submission and has agreed to all information as it was submitted. I confirm that disclosures of all co-authors were declared.

37
EXPLORING THE USE OF SMALLER ELIGLUSTAT DOSES FOR IMPROVED DOSING FLEXIBILITY AND SAFETY

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BACKGROUND: Eliglustat, an oral inhibitor of glucosylceramide synthase, is indicated for long-term treatment of adults with type 1 Gaucher disease. It is extensively metabolized by CYP2D6, and to a lesser extent by CYP3A4. Patient’s CYP2D6 metabolizer status and use of concomitant medications are important determinants of eliglustat dose and eligibility. Co-administration with a strong metabolism inhibitor is contraindicated owing to safety concerns, restricting the use of eliglustat. We hypothesize that smaller eliglustat doses (42mg, 21mg) would be safer alternatives to the regular dose (84mg) in certain drug-drug interaction (DDI) scenarios.

AIMS: The aims of this study were to (1) develop and validate eliglustat physiologically based pharmacokinetic (PBPK) model with and without drug interactions, and (2) explore the potential for safety and dosing flexibility through the potential use of smaller doses.

METHODS: Eliglustat pharmacokinetic data (with or without interaction drugs) in healthy adults were obtained from literature and used for development and validation of PBPK model. Based on the current labeling guidance, DDI scenarios where eliglustat is contraindicated were identified. Simulations were conducted to address those scenarios and to evaluate the feasibility of using smaller doses.

RESULTS: The developed model predicted single dose and steady-state peak plasma-concentration (Cmax) and area under the curve (AUC) of eliglustat within 50-150% of the observed values, indicating acceptable model performance. Our simulations demonstrated that smaller doses
maintained eliglustat exposures within the outlined safety margin (Cmax < 250ng/mL, AUC0-24h < 1100 ng*h/mL) in CYP2D6 intermediate and poor metabolizers when administered with a strong 3A4 inhibitor.

CONCLUSIONS: The present study displayed the potential safety benefits of using smaller doses of eliglustat in interaction situations, without the need to stop treatment in patients with certain CYP2D6 metabolizer phenotypes. Although smaller doses are not commercially available, current eliglustat sponsor or any generic competitor may choose to develop those, making it a near-term possibility.

DISCLOSURE FOR ALL AUTHORS: RVK has received grants from Sanofi, Pfizer Inc. and the National Institutes of Health. NJW is a speaker and consultant for Genzyme, a Sanofi Company that is the manufacturer of eliglustat. NIJW serves as a consultant with Pfizer Inc. SAS, SC, MAK have nothing to disclose in reference to this work.

40 MEASUREMENT OF GLUCOSYLCERAMIDE SYNTHESIS IN LIVING CELLS WITH ISOTOPE-ENCODED SPHINGOSINE PRECURSOR

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BACKGROUND: Glycosphingolipids fulfill diverse functions in cells. Abnormalities in their metabolism are associated with specific pathologies and consequently their pharmacological modulation is considered as therapeutic avenue. Employing synthesized sphingosine and sphinganine containing five 13C atoms, we developed a method to monitor de novo synthesis of glucosylceramide (GlcCer) by glucosylceramide synthase (GCS). To assess in vivo IC50 values of therapeutically considered inhibitors (iminosugars and ceramide mimics) in intact cultured cells.

Aims: To assess in vivo IC50 values of therapeutically considered inhibitors (iminosugars and ceramide mimics) in intact cultured cells.

Methods: Feeding of cells with 13C encoded sphingosine (or 13C-sphinganine); lipid extraction; followed by quantification of lipids by LC-MS/MS.

 RESULTS: We show that the feeding of cells with isotope-labelled precursor sphingosine combined with LC-MS/MS analysis of (glio)spingolipids allows the accurate determination of IC50 values of therapeutically considered inhibitors (iminosugars and ceramide mimics) of GCS in intact cultured cells.

Cells incubated with 1 µM 13C-sphingosine for 3 hours allowed optimal, accurate assessment of formation of 13C-GlcCer in fibroblasts. At these conditions no other enzymes were found to impact 13C-GlcCer levels. Acquired data were comparable to those obtained with an earlier employed method using artificial NBD-ceramide feeding of cells. IC50 values for Eliglustat were 24 nM and 6 nM, for Miglustat 25 µM and 55 µM, and for AMP-DNM 150 nm and 340 nM; with NBD-ceramide and 13C-sphingosine feeding, respectively. In principle, the method can be expanded to measure other enzyme activities in the pathway in situ; for example, activities of lactosylceramide synthase, Gb3 synthase as well as in situ activity of lysosomal enzymes.

CONCLUSIONS: A novel method has been developed to determine the real life synthesis of GlcCer and the impact of GCS inhibitors on the flux.

DISCLOSURE FOR ALL AUTHORS: No disclosure to submit.

68 INVESTIGATION INTO THE PATHOPHYSIOLOGY OF GBA1-ASSOCIATED PARKINSON DISEASE USING ORGANELLE-SPECIFIC PROTEOMICS

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BACKGROUND: Mutations in GBA1 are a common genetic risk factor for Parkinson disease (PD). GBA1 encodes the lysosomal enzyme glucocerebrosidase (GCase), which hydrolyzes its lipid substrate glucosylceramide to glucose and ceramide. Gaucher disease (GD) occurs when there are biallelic pathological mutations in GBA1 leading to aberrant GCase activity and lipid substrate accumulation. To better understand the molecular perturbations leading to GBA1-associated PD, we profiled the proteomes of lysosomes and mitochondria isolated from iPSC-derived dopaminergic neurons generated from patients with biallelic GBA1 mutations. iPSC lines were made from fibroblasts from patients with both GD and PD (GBA1 N370S/N370S). Using CRISPR-Cas9 genome editing approaches we inserted into the CLYBL safe-harbor locus a transgene cassette expressing Tmem192-3xHA, a lysosomal transmembrane protein, and TOM20-2Strep, an outer mitochondrial membrane protein. We developed an optimized workflow for the rapid purification of both organelles, sequentially using anti-Strep and anti-HA antibody-conjugated magnetic beads. Characterization of the mitochondrial and lysosomal fractions using Western blot analyses confirmed the successful enrichment of both organelles without significant contamination from other intracellular contents. Preliminary mass spectroscopy analysis of the enriched fractions revealed the presence of luminal and peripheral proteins associated with the appropriate organelles. Additionally, to compare differences caused by mutations in GBA1, we created isogenic GBA1 iPSC lines by knocking out the GBA1 gene and converting the N370S mutation back to wild type. The rapid purification of mitochondria and lysosomes provides a novel means to evaluate the molecular changes within the two organelles induced by mutant GBA1. The method uniquely enables quantitative mass-spectrometry-based proteomic analyses at the organelle level, and more accurately reflects lysosomal and mitochondrial alterations contributing to PD pathogenesis.
DISCLOSURE FOR ALL AUTHORS: Work related to the Chen abstract was funded in part by the MJFF ASAP program.

71 MEASUREMENT OF GLUCOSYLCERAMIDE SYNTHESIS IN LIVING CELLS WITH ISOTOPE-ENCODED SPHINGOSINE PRECURSOR

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BACKGROUND: Glycosphingolipids fulfil diverse functions in cells. Abnormalities in their metabolism are associated with specific pathologies and consequently their pharmacological modulation is considered as therapeutic avenue. Employing synthesized sphingosine and sphinganine containing five 13C atoms, we developed a method to monitor de novo synthesis of glucosylceramide by glucosylceramide synthase (GCS).

AIMS: To assess in vivo IC50 values of therapeutically considered inhibitors (iminosugars and ceramide mimics) in intact cultured cells.

METHODS: Feeding of cells with 13C encoded sphingosine (or sphinganine); lipid extraction; quantification of lipids by LC-MS/MS.

RESULTS: We show that the feeding of cells with isotope-labelled precursor sphingosine combined with LC-MS/MS analysis of (glyco)sphingolipids allows the accurate determination of IC50 values of therapeutically considered inhibitors (iminosugars and ceramide mimics) of GCS in intact cultured cells.

Cells incubated with 1 µM 13C-sphingosine for 3 hours allowed optimal, accurate assessment of formation of 13C glucosylceramide (GlcCer) in fibroblasts. At these conditions no other enzymes were found to impact 13C GlcCer levels. Acquired data were comparable to those obtained with an earlier employed method using artificial NBD-ceramide feeding of cells. IC50 values for Eliglustat were 24 nM and 6 nM, for Miglustat 25 µM and 55 µM, for AMP-dnm 200 nm and 340 nM with both methods respectively. In principle, the method can be expanded to measure other enzyme activities in the pathway in situ; for example, activities of lactosylceramide synthase, Galβ3 synthase as well as in situ activity of lysosomal enzymes.

CONCLUSIONS: A novel method has been developed to determine the real life synthesis of GlcCer and the impact of GCS inhibitors on the flux.

72 GLUCOSYLATION OF RETINOL AND DELTA-TOCOPHEROL. FIRST INVESTIGATIONS.

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BACKGROUND: In our laboratory it has been discovered that both cellular retaining β-glucosidases are able in the test tube and in vivo to transglucosylate cholesterol using glucosylceramide (GlcCer) as sugar donor. GlcChol is ubiquitously present in human tissues. Physiologically, GBA2 seems to act as synthetic enzyme and lysosomal GBA1 (glucocerebrosidase) as hydrolase releasing glucose from GlcChol. We have embarked on a search for additional glycosylated metabolites (glycolites) formed by transglucosylation. Considered candidates for transglucosylation besides sterols are retinol and tocopherol.

AIMS: Studied was glucosylation of retinol and delta-tocopherol by GBA1 and GBA2.

METHODS: Incubation of compounds with enzymes GBA1, GBA2 or GBA3 and in the presence of the sugar donor 4MU-β-glucoside and in some cases 4MU-β-xyloside. Occasionally, GlcCer was also used as sugar donor. Lipids were subsequently extracted. LC-MS/MS was used to quantify the lipids of interest.

RESULTS: We observed that in vitro GBA1 and GBA2 are able to glucosylate retinol (vitamin A). GBA1 was also found to be able to generate xylosylated retinol. Hydrolysis of Glc-retinol to retinol and glucose was observed for GBA1. GBA2 was found to form glycosylated retinol, but not xylosylated retinol. GBA3 was found to form in vitro Glc-, Gal- and Xyl-retinol. Likely, it will hydrolyze these as well, as was demonstrated for Glc-retinol. In the case of delta-tocopherol, initial experiments revealed that GBA1 is able to glucosylate it.

CONCLUSIONS: We were able to confirm an old report by Glew and collaborators that retinol can in vitro be glucosylated by GBA1. We extended the observation to glucosylation of retinol by GBA2, the physiologically main synthetic transglycosidase. Demonstration of natural occurrence of Glc-retinol is most likely hampered by the lability of the metabolite. Freshly obtained plasma samples will be required to establish abnormalities in Glc-retinol and Glc-tocopherol in Gaucher patients.

OTHER

25 TRANSITION OF GAUCHER DISEASE (GD) PATIENTS FROM PAEDIATRIC TO ADULT SERVICES-RESULTS OF TWO INTERNATIONAL SURVEYS

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BACKGROUND: Transition is a process of addressing the complex needs of young adults when they move from child-centred to adult services.

AIMS: The aim of this international and multi-centre project was to evaluate the current transition clinic service in various countries, and to learn about its challenges from both healthcare professionals’ (HCP) and patients’.

METHODS: Two survey monkeys were designed by members from the IWGGD supportive care working group and IGA members. Links were shared with healthcare professionals looking after patients diagnosed with GD as well as GD patients. The project was registered as a service evaluation project.

RESULTS: Among 26 HCPs, most clinicians work in metabolic medicine (38%), followed by haematology, genetics, internal medicine and others. Respondents were from 4 continents. Only 40% of HCPs work in transition clinics and among those, 46% have transition coordinator and most (78%) have a transition protocol. In most centres, the transition starts at 18 years of age. The main challenges of transition are: problems with coordination of GD patients’ complex care (multidisciplinary), limited funding and lack of interest among adult physicians. 45 patients from 6 continents remained under specialist care (84%); metabolic medicine, haematology, clinical genetics, internal medicine and gastroenterology. The majority of respondents were adults.

CONCLUSIONS: There is no standardized model of care during the transition process of GD patients across the world. The best approach seems to be to advocate for principles that would facilitate an effective transition such as autonomy, adherence to the therapy, self-directed management, maintaining relationship with the hospital staff and ability to consent. It is important to develop good clinical practice guidance to help centres establish protocols for transition, reduce local diversities and encourage the concept of transition clinics. Coordination of transition care is the key to manage patients’ expectations and empower young adults.

DISCLOSURE FOR ALL AUTHORS: None

26 RESULTS FROM A DELPHI SURVEY: RECOMMENDATIONS ON THE FOLLOW-UP OF PATIENTS WITH GAUCHER DISEASE IN SPAIN

P. Giraldo 1, M. Morales 2, M. Andrade-Campos 3

BACKGROUND: Management of Gaucher Disease (GD) is challenging because of its wide genotypic and phenotypic variability. In addition, the appearance of effective therapies for GD1 (enzyme replacement therapy, ERT and later substrate reduction therapy, SRT) has shifted the paradigm of care, and recommendations for GD management should now include strategies for patients without treatment, long-term treated patients, reproductive health, and transition of care from children to adults.

AIMS: To present the results of a Delphi survey on the follow-up of GD patients in specific clinical situations in Spain.

METHODS: Sixteen face-to-face meetings with experts were held to discuss daily clinical practice and to identify controversies regarding the management of GD. With this information, a questionnaire with 93 recommendations for different clinical scenarios was designed, and a Delphi survey among 86 physicians with experience in GD patient care was conducted.

RESULTS: The inquiry shows consensus in 73 out of the 93 items. Recommendations on follow-up of adult and pediatric patients were in line with current guidelines, and underscored the importance of a patient-tailored approach. For the follow-up of stable patients receiving long-term treatment, consensus was reached on the importance of multidisciplinary team collaborating with the expert physician in GD, and specialists with GD knowledge (nurses, primary care, specialized radiologists, etc.), when required. Consensus was reached on the frequency of follow-up depending on the disease evolution time, whether or not patients received treatment, clinical disease stability, and specific recommendations for pregnant patients were also stated. Experts stressed on the importance of asking about symptoms reflecting quality of life such as pain, use of analgesics, antidepressants, etc. Lastly, recommendations on how to adapt GD management during a COVID-19 pandemic were collected.

CONCLUSIONS: This expert consensus will help decision-making during the management of GD in specific clinical scenarios.

DISCLOSURE FOR ALL AUTHORS: I am submitting this abstract with the intent of attending the IWGGD 2022 Scientific meeting and presenting. I confirm each co-author has been informed of this abstract submission and has agreed to all information as it was submitted.

27 TWO YEARS OF VENGLUSTAT COMBINED WITH IMIGLUCERASE SHOWS CONTINUED POSITIVE EFFECTS ON
**NEUROLOGICAL FEATURES AND BRAIN CONNECTIVITY IN ADULTS WITH GAUCHER DISEASE TYPE 3**

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**BACKGROUND:** Venglustat is a brain-penetrant glucosylceramide synthase inhibitor under investigation for treatment of Gaucher disease type 3 (GD3). After 1 year of venglustat+imiglucerase in the Phase 2 LEAP trial (NCT02843035/Sanofi), target venglustat concentrations were maintained in CSF, median glucosylceramide and glucosylsphingosine in plasma and CSF decreased markedly with favorable safety and tolerability. In addition, ataxia scores improved and brain volume increased in patients with adequate venglustat exposure, and fMRI showed stronger connectivity between brain regions at Weeks 26 and 52 relative to baseline.

**AIMS:** Evaluate 2-year treatment outcomes in LEAP.

**METHODS:** The Phase 2 LEAP trial evaluated oral venglustat (15-mg daily) + intravenous imiglucerase (usual dose) in 11 adults with GD3 who had received imiglucerase for ≥3 years and achieved non-neurological therapeutic goals before enrollment. Primary endpoints were safety, tolerability, and changes in glucosylceramide and glucosylsphingosine concentrations in plasma and CSF during 1 year of treatment. Secondary endpoints included neurological assessments, volumetric and functional brain MRI, and systemic disease measures. All patients continued treatment for an additional year.

**RESULTS:** There were no deaths or adverse events leading to treatment discontinuation. Systemic manifestations remained stable. One patient (Patient 9) had low-to-undetectable venglustat exposure after Week 26. In the other 10 patients, decreases in plasma glucosylsphingosine at 1 year (mean % change from baseline: -53.3% ± 13.5%) continued at 2 years (-59.0% ± 11.5%). On the Scale for Assessment and Rating of Ataxia score, 91% of patients with ataxia at baseline improved or had no worsening at 1 and 2 years. Brain atrophy on MRI partially reversed from year 1 to 2, and connectivity across key brain regions (resting state networks) on functional MRI was enhanced among the 10 patients with venglustat exposure.

**CONCLUSIONS:** During two years of treatment, venglustat+imiglucerase showed favorable safety and tolerability outcomes and salutary neurological improvements were maintained.

**DISCLOSURE FOR ALL AUTHORS:** IB, RZ, PM, and DC are employees and/or stockholders at Sanofi. TC is a principal investigator on the venglustat LEAP and AMETHIST trials and the former eliglustat ENCORE trial—all sponsored by Sanofi. He reports receiving honoraria, travel reimbursement and grant/research support from Shire (now Takeda) Pharmaceuticals.

**38 TRANSGLUCOSYLATION OF DESMOSTEROL AND SOME OTHER STEROLS. RELEVANCE FOR GAUCHER DISEASE.**

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**BACKGROUND:** We earlier discovered that glucosylated cholesterol (GlcChol) is formed by the two cellular β-glucosidases GBA1 and GBA2 through a transglucosylation of cholesterol. GlcChol is also a substrate of GBA1, being cleaved into cholesterol and glucose. These findings prompted us to study whether related sterols are also subject to transglucosylation and whether formed glucosylated products are subject to breakdown by GBA1.

**AIMS:** Assessment of the metabolism by GBA1 and GBA2 of desmosterol, 7-dehydrocholesterol and its product D3. Attention for the formation of glucosylated compounds by transglucosylation as well as their hydrolytic breakdown.

**METHODS:** Experiments were performed with pure or enriched enzyme. Investigations were conducted with spleens and plasma samples of type 1 GD patients. LC-MS/MS with newly synthesized 13C isotope-encoded standards was used to quantify the lipids of interest.

**RESULTS:** We observed that in vitro GBA1 and GBA2 both can convert desmosterol (Desm) into GlcDesm. In spleens of GD patients, GlcDesm is elevated. Consistent with this, GlcDesm was found to be degraded by pure GBA1 in vitro. Next, we investigated 7-dehydrocholesterol (7DHCh) in a similar manner. Again, GBA1 and GBA2 were able in vitro to generate Glc7DHCh. Degradation of synthesized Glc7DHCh by GBA1 was observed. Physiologically, 7DHCh is converted to cholecalciferol (D3) by UVB. We examined therefore next also D3. It was found to be transglucosylated GlcD3 by GBA1 and GBA2. Synthesized Glc7DHCh was found to be degraded by GBA1, but not synthesized GlcD3. Only low levels of Glc7DHCh and GlcD3 were detected in GD and corresponding control materials.

**CONCLUSIONS:** The abnormality in desmosterol metabolism in Gaucher patients is of potential interest given the well-known anti-inflammatory action of desmosterol. Abnormalities in desmosterol might contribute to the reported chronic low-grade inflammation for Gaucher patients. Likewise, the noted glucosylation of 7DHCh and D3 given reported vitamin D abnormalities in Gaucher patients.

**DISCLOSURE FOR ALL AUTHORS:** Acknowledgement. Sphinx is thanked for the collaboration.
GAUCHER DISEASE HISTORY GROUP

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1International Gaucher Alliance (Netherlands), 2Iwggd (Netherlands), 3International Gaucher Alliance (North Macedonia, Republic of), 4Gaucher Community Alliance (United States), 5International Gaucher Alliance (Canada)

BACKGROUND: In 1882, Gaucher Disease entered the world of science and medicine. Its history reflects the history of areas like medicine, biochemistry, radiology, genetics, and pharmaceutical endeavor - and of patients around the world, as well as their next of kin and the societies they lived in.

It is a history worth documenting, not least to enable (future) historic research.

Medical and scientific advances are well documented in literature. An abundance of relevant documentation etc. remains to be secured. Examples are images, audio-visual content, and communication like letters. The custodians of research collections by preference are archives, but these are hesitant in collecting the materials at hand.

AIMS: 1. Securing and mapping relevant material for future historic research. 2. Transferring to next generations main insights and lessons learned from the history of Gaucher disease in medicine and science, the lives of patients, and third parties. 3. Gain insight into mechanisms that contributed to success in research and innovation. 4. Gain insight into the history of GD, especially of patient advocacy and cooperation. One idea is to publish an encompassing volume on the multidisciplinary history of GD in 2032, 150 years after Ernest Gaucher attached his name to the disease that brought us together.

METHODS: An informal platform has been established of individuals. They participate on their own account, not on behalf of any organization. In the group, thoughts and experiences are shared.

RESULTS: The group maintains an inventory of relevant collections, including a collection of filmed interviews with key persons in GD History, and is in contact with several relevant organizations including the NIH History & Stetten Museum and the European Association for the History of Medicine and Health.

CONCLUSIONS: The GD History Group, just started, welcomes any thoughts and support. Anyone interested is welcome to join.

DISCLOSURE FOR ALL AUTHORS: The authors do not have any conflicts of interest.

TOWARDS GENETIC MODIFIERS OF GAUCHER DISEASE – GWAS IN RARE METABOLIC DISEASES

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BACKGROUND: Genetic modifiers, i.e. variants in the genome which are not the disease-causing ones, may have a protective or aggravating affect on the disease severity, which results in the phenotypic variability among patients with the same disease. In this work, we aim to identify such genetic modifiers in Gaucher Disease (GD) by applying genome wide association studies (GWAS).

The classification of GD patients into type I and type II or III is based on the lack or presence of neurological involvement. Here we propose a stratification of GD patients which is based both on genetic and clinical parameters. First, the patients were stratified according to the GBA variants and zygosity. Then, the patients were stratified according to their age, Lyso-Gb1 biomarker levels, and platelet counts. In addition, specific phenotypes, such as splenectomy and hepatomegaly, were linked with more severe clinical manifestation.

In order to identify genetic modifiers, which contribute to disease severity, we applied a GWAS on whole genome sequencing (WGS) data from 240 GD patients. We compared two groups of patients, mild and severe, and used several models to determine the statistical association of coding and non-coding variants with the disease severity. Furthermore, we applied a gene-collapsing strategy and tested for statistical associations on the gene level.

We identified 37 significant (P< 8×10−8) variants spanning over 17 genes, 16 of them on chromosome 1 and in various distances from the GBA gene. Six of the variants were enriched in the severe cohort, and the rest were enriched in the mild cohort. Most of the variants were located in non-coding regions such as 3’ prime and 5’ prime UTR, splice regions as well as intronic regions. Further investigations are required to estimate if and how any of these variants account for the variability in disease severity.

DISCLOSURE FOR ALL AUTHORS: No disclosure to submit.

PULMONARY INVOLVEMENT IN PATIENTS WITH GAUCHER DISEASE IN A BRAZILIAN REFERENCE CENTER

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BACKGROUND: Gaucher disease (GD) is an autosomal recessive condition caused by the defective activity of the
The safety and efficacy of rapid 10-minute infusions of high dose velaglucerase-alfa in naïve patients with GD.

AIMS: To present the 24-month results of rapid 10-minute infusion of high dose velaglucerase-alfa in naïve patients with GD.

METHODS: Consecutive naïve patients (not previously treated or at least one year off GD-specific therapy) were eligible for this investigator-initiated research. All patients received bi-weekly infusions of 60 unit/kg BW velaglucerase-alfa; the infusion rate was gradually reduced in the hospital, followed by home infusions (figure). Each infusion was followed for safety. Efficacy parameters were assessed every three months.

RESULTS: We enrolled 15 patients (77% males) at a median age (range) of 40 (10-72) years. Thirteen patients were never treated, and two patients were off-ERT for over one year. Ten-minute rapid infusions were well tolerated with no reported related severe or non-severe adverse events (AEs). Two patients reported a non-related SAE and another a non-related AE. In 3 patients the infusion rate was increased back to 30 or 60 minutes (2 due to suboptimal response and one due to AE).

Two patients dropped out due to unwillingness to attend follow-up visits during the COVID-19 pandemic. All 13 remaining patients reached the 24-month time-point. The platelet counts increased by a median (range) of 68.38%(12.5%-300%) and the lyso-GB1 levels decreased by 62.6%(32.9%-89.9%).

CONCLUSIONS: Based on the safety and efficacy of rapid 10-minutes in both naïve and previously treated patients, we have submitted a request to the Israel ministry of health for approval of the 10-minute infusion of velaglucerase-alfa (following a stepwise time reduction). Whether similar results could be achieved in other ERT would need to be studied separately.

DISCLOSURE FOR ALL AUTHORS: The SZMC Gaucher Unit receives support from Sanofi/Genzyme for participation in the ICCG Registry, from Takeda for the GOS Registry, and Pfizer for TALIAS. The Unit also receives research grants from Takeda, Pfizer, Sanofi/Genzyme, and Centogene. M.B.-C., D.F., T.D., and M.I. have no conflict of interest to declare. S.R.-V. receives grant/research support, honoraria, and travel fee from Takeda, Pfizer, and Sanofi/Genzyme. C.C. is an employee of Centogene GmbH. A.R. is the founder and was the CEO of Centogene GmbH during the study. A.Z. receives honoraria from Takeda, Pfizer, and BioEvents and consultancy fees from Takeda, and Prevail therapeutics. This study was supported by a grant from Takeda.
**PREIMPLANTATION GENETIC TESTING (PGT) FOR PARKINSON DISEASE (PD) RISK REDUCTION: EXPANDING OF APPLICATIONS FOR EMBRYO SELECTION**

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**BACKGROUND:** PGT is practiced worldwide, allowing preventing transmission of a growing numbers of genetic conditions. Social and ethical considerations of justified applications for PGT are in an ongoing debate.

**METHODS:** Pathogenic variants in the glucocerebrosidase (GBA) gene, causing Gaucher disease (GD), have emerged as a primary risk factor for PD in patients and carriers. Genotype-phenotype correlations exist between different GBA mutations and the risk to develop PD: Mild variants increase the risk of developing PD by 2.2-fold while severe variants increase the risk of developing PD by 10.3-fold. As regard to age of onset of PD: carriers of severe variants were diagnosed at 53.1 years compared to carriers of mild variants diagnosed at age 58.1 years in average.

PGT counseling was given to a 32 year old women compound heterozygote of N370S (mild variant) and 84GG (severe variant) whose mother (an 84GG carrier) died of early-onset PD with severe cognitive decline (possibly LBD). GBA sequencing of her spouse was negative. Since the couple needed IVF anyway they discuss selection for N370S carrier embryos with a lower risk of developing PD, compared to 84GG carrier embryos. Eight embryos were sampled for the familial GBA variants in conjunction with chromosomal analysis for aneuploidy: Four were carriers for 84GG mutation; 1 aneuploid; 1 did not provide a conclusive result; 2 were N370S carriers and thus transferrable.

**CONCLUSIONS:** This case report demonstrates the expansion of PGT for molecular disorder (PGT-M) use for risk reduction for late onset conditions. These novel indications for PGT-M will increase the numbers of subjects who would be candidates for PGT-M. The medical and bioethical consideration of these cases should be acknowledged by professional community and discussed with couples in the genetic counseling setting. Guidelines for PGT usage for preventing genetic traits, increased risk for late onset disorders should be implemented.

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**DISCLOSURE FOR ALL AUTHORS:** Conflict of Interest Statement: The authors have no ethical conflicts to disclose.

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**TYPE 1 GD**

**FROM PRODROMAL SYMPTOMS TO THE DIAGNOSIS OF PARKINSON’S DISEASE: A CASE REPORT OF A GAUCHER DISEASE TYPE 1 PATIENT WITH HYPOSMIA.**

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**BACKGROUND:** Parkinson’s disease (PD) is the second most common neurodegenerative disease. The classic motor symptoms of PD may be preceded by many non-motor symptoms (NMS), such as hyposmia, REM sleep behavior disorder, constipation, cognitive impairment, and depression. Population studies have identified mutations in GBA1 as the main risk factor for idiopathic PD.

**AIMS:** This is a case report aiming to raise awareness for the importance of the evaluation of NMS of PD in adult patients with Gaucher Disease type 1 (GD1).

**METHODS:** The evaluation of NMS was assessed in 2018 and 2021 in a cohort of adult patients with GD1 (n=23). Cognition was evaluated by the Montreal Cognitive Assessment (MoCa), daytime sleepiness by the Epworth Scale, depression by Beck’s Inventory (BI), constipation by the Unified Multiple System Atrophy Rating Scale (UMSARS), and REM sleep behavior disorder by the single-question screen. Hyposmia was assessed with Sniffin’ Sticks (SS).

**RESULTS:** This is a female patient (genotype is N370S/RecNciI), currently with the age of 66. The patient started treatment with enzyme replacement therapy at the age of 42 due to anemia, thrombocytopenia, and splenomegaly. In the 2018 evaluation the patient had four NMS detected: Depression (BI=17), constipation (UMSARS = 2), low cognition (MoCa =22) and hyposmia (SS= 4). The patient had the highest number of NMS at the baseline and the lowest SS score in our cohort (cohort mean SS score: 11±1.9). In 2021 follow up she was diagnosed with PD due to bradykinesia and tremor. PD genetic panel only included a VUS in VPS13C not deemed relevant.

**CONCLUSIONS:** The patient with the highest number of NMS in 2018 was the one that developed PD after 4 years. Hyposmia appears to be the most specific NMS of PD in our cohort. This study corroborates the importance of longitudinal follow-up for these patients.

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**DISCLOSURE FOR ALL AUTHORS:** Conflict of Interest
CORRELATION BETWEEN SUBCORTICAL BRAIN STRUCTURES VOLUMETRY AND NON-MOTOR PARKINSON SYMPTOMS IN GAUCHER TYPE 1 DISEASE

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BACKGROUND: Gaucher type 1 is the non-neuronopathic form of Gaucher Disease (GD), caused by biallelic pathogenic GBA1 gene. GBA1 is considered a risk factor for Parkinson’s Disease (PD). The typical motor symptoms of PD can be preceded years before by non-motor symptoms (NMS) such as: hyposmia, rapid eye movement (REM). Magnetic resonance imaging (MRI) with high-resolution protocols allows precise volumetry of brain structures.

AIMS: This study sought to analyze the relationships between NMS and deep cortical structures volumes in GD1 patients from Southern Brazil.

METHODS: Automatized subcortical volumes were obtained using FreeSurfer software from MRI exams of patients with genetically confirmed GD1. NMS were evaluated 5 years later, including cognition evaluation by Montreal Cognitive assessment (MoCa), daytime sleepiness by Epworth Sleepiness Scale (ESS), depression by Beck Inventory (BDI), and hyposmia by Sniffin’ Sticks (SSI).

RESULTS: Eighteen GD1 patients (female=10) were included with a mean age of 36.3±14.7 years (range, 22–60) at the time of MRI. Eleven patients presented at least one NMS; daytime sleepiness and cognition were the most frequent (n=7). One patient had confirmed PD 7 years after the MRI. There was moderate negative correlation between BDI and volumetry of right (R=-0.713, p=0.001) and left caudate (R=-0.722, p=0.001), right (R=-0.817, p=0) and left putamina (R=-0.717, p=0.001), right amygdala (R=-0.76, p=0) and right pallidum (R=-0.76, p=0); moderate positive correlation between MoCa and right (R=0.718, p=0.003) and left thalami (R=0.72, p=0.002) volumetry; and moderate negative correlation between ESS and left caudate volumetry (R=-0.767, p=0.001). There was also mild negative correlation (p<0.05) between BDI and volumetry of left amygdala and both thalami, and between ESS and left hippocampus and right putamen volumetry, and mild positive correlation between MoCa and left amygdala volumetry and between ESS and right pallidum volumetry.

CONCLUSIONS: These results suggest that volumetric subcortical changes may precede some NMS in GD1 patients.

DISCLOSURE FOR ALL AUTHORS: The authors report no disclosures.
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