

Dear colleague,

Welcome to the 10th EWGGD Meeting. This year's meeting will be held from June 28th to 30th, 2012 at the Novotel Paris Tour Eiffel in the heart of Paris, city of light, knowledge and culture.

The quality of the scientific content at the EWGGD meetings attracts an increasing number of participants with each edition. Our delegates are recognized experts in their fields, committed to both clinical and basic science in Gaucher disease.

Representatives from the European Gaucher Alliance, the patients' association and a key partner of our conference, will share their experience and questions with the scientific community.

The medical community eagerly anticipates the results of basic science, clinical trials and registries highlights, to be announced during the meeting. Updated guidelines for the management of Gaucher disease will be released, taking into account the experience of different teams in various countries.

The Paris conference will offer a number of innovations in 2012. One of the new features is a practical session and workshop, dedicated to nurses and paramedics with the objective of better integrating their crucial role in patient management through medical education, based on new practices such as home therapy.

A special part of the programme will be oriented towards the emerging medical community, our younger colleagues. We aim to assist them in the acquisition of skills and experience through a dedicated workshop highlighting both typical and unusual clinical cases, presented and discussed in-depth.

We look forward to meeting you in Paris.

Best regards,

The Organising Committee

Scientific Committee

Stephan vom Dahl	Prof. Dr. Med, St. Franziskus Hospital Köln, Germany
Mia Horowitz	PhD, Department of Cell Research and Immunology Tel Aviv, University
Helen Michelakakis	PhD, Institute of Child Health Athens, Greece
Nadia Belmatoug	MD, Referral Center for Lysosomal Diseases, Beaujon Hospital Clichy, France

Executive Committee

Chairperson

Stephan vom Dahl	Prof Dr. Med, St. Franziskus Hospital Köln, Germany
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Vice Chairperson

Helen Michelakakis	PhD, Institute of Child Health Athens, Greece
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Members

Hans Aerts	Prof, Academic Medical Centre University of Amsterdam
Nadia Belmatoug	MD, Referral Center for Lysosomal Diseases, Beaujon Hospital Clichy, France (EWGGD Host 2012)
Marieke Biegstraaten	MD, PhD Academic Medical Center, University of Amsterdam
Timothy Cox	Prof, Addenbrooke's Hospital, Cambridge, UK
Carla Hollak	Prof, Academic Medical Centre University of Amsterdam
Jeremy Manuel, OBE	European Gaucher Alliance (EGA)

Invited speakers

Johannes M. F.G. Aerts

Department of Medical Biochemistry, Academic Medical Center, UvA, Amsterdam

The laboratory of Hans Aerts is internationally recognized as one of the leading academic research laboratories on glycolipid biology in relation to glycolipid storage disorders (Gaucher, Fabry). The Aerts group identified several enzymatic activities (non-lysosomal glucosylceramidase, or GBA2; human chitotriosidase as a marker in Gaucher disease) and recently unearthed the involvement of glucosylceramide synthase, in conjugation with a high-fat diet, in the onset of diabetes mellitus type II and metabolic syndrome. His research group now focuses on fundamental research on glycosphingolipids in relation to storage disorders, employing novel chemical tools to visualize enzymes and monitor metabolism in vivo. Development of novel therapy approaches for (neuronopathic) Gaucher disease is actively pursued at the Aerts laboratory. His work is supported by an European Research Council Advanced Grant.

Ilan Yaron

Dr. Yaron Ilan is the Director of the Department of Medicine at Hebrew University Hadassah Medical Center.

Between 2002 to 2009 he served as the Vice Dean of the Hebrew University-Hadassah Medical School.

Between 1995 and 1997, Ilan engaged in postgraduate studies at the Liver Research Center of Albert Einstein College of Medicine in New York and served on the staff of the Division of Liver Transplantation and the Liver Unit at Mount Sinai Medical Center. Between 2007-2008 he worked at the Brigham and Women Hospital in Boston, and at Harvard Institute of Medicine in Boston.

Dr. Ilan served as the President of the Israel Liver Association from 2005 to 2008.

Dr. Ilan is a world-renowned scientist in the fields of internal medicine, immunology, and liver diseases, with more than 230 peer-reviewed publications to his name. Dr. Ilan is an inventor of more than 40 patents, and is the inventor of a number of drugs being developed by pharmaceutical companies. He has a significant biotech industry experience as a founder of several start up companies.

Pierre Lafforgue

He is at the head of the department of rheumatology, at Sainte Marguerite University Hospital in Marseille, France.

He published in the field of pathophysiology and natural history of avascular necrosis of bone.

He is following patients with Gaucher disease.

Liz Morris

Lysosomal Disorders Unit Box 135, Addenbrookes Hospital, Hills Road, Cambridge.
CB2 0QQ UK

Liz Morris is a specialist nurse in Lysosomal Storage Disorders at Addenbrooke's Hospital in Cambridge, UK. She qualified in 1989 and has worked as a Specialist Nurse for adult patients with Gaucher Disease since 1996, setting up the first UK national Gaucher centre. She administered the first Ceredase & Cerezyme infusions in the UK and similarly took part in the initial clinical trials of Zavesca. She is responsible for managing the Lysosomal Storage Disorders service at Addenbrooke's, alongside her clinical and research roles. Liz lives with her family just outside Cambridge.

Wout Timmerman

Is a patient with Gaucher disease.

He is married with 2 children.

He has been working with mental retarded people, and now a very involved volunteer.

He was a caregiver with some medical background.

He has been the chairman of the Dutch Patient Organisation for Gaucher disease during 8 years.

He is for the VKS contact for the Gaucher Diagnosis group in the Netherlands.

He is member of the EGA.

Roscoe Brady

Dr. Brady attended the Pennsylvania State University and received an M.D. degree from Harvard Medical School. After interning at the Hospital of the University of Pennsylvania, he was a post-doctoral fellow in the Department of Physiological Chemistry at the University of Pennsylvania School of Medicine and a fellow in clinical medicine in the Department of Medicine. Following two and one-half years on active duty in the U.S. Naval Medical Corps, he joined the National Institutes of Health where he was Chief of the Developmental and Metabolic Neurology Branch from 1972 to 2006. He is now a scientist emeritus at the National Institutes of Health, Bethesda, MD. He received the Gairdner International Award, the Lasker Foundation Clinical Medical Research Award, the Kovalenko Medal from the National Academy of Sciences USA and the National Medal of Technology and Innovation. He is a member of the National Academy of Sciences, USA and a member of the Institute of Medicine of the National Academy of Sciences. Dr. Brady investigated the enzymatic and molecular bases of hereditary metabolic disorders and developed effective therapies for patients with these conditions.

PROGRAMME

Thursday, June 28 2012

- 08.30–08.45** **Welcome Address**
Nadia Belmatoug, France
Stephan vom Dahl, Germany
- 08.45-10.00** **Session 1: Opening session**
Chair :Marie T. Vanier, France
- 08.45-09.45** **Joseph Tager Memorial Lecture**
A tribute to Joseph Tager : On the value of fundamental laboratory research:
Novel chemical tools to study Gaucher disease pathophysiology and therapy.
Han Aerts, The Netherlands
- 09.45-10.00** **In memory of Rene Heitner, South Africa** *Ari Zimran, Israel*
In memory of Ursula Rudat, Germany *Pascal Niemeyer, Germany*
In memory of Milan Elleder, Czech Republic *Marie T. Vanier, France*
- 10.00-10.30** **Coffee break**
- 10.30-11.45** **Session 2 : Type 3 Gaucher disease**
Chairs : Bruno Bembi, Italy; Frédéric Sedel, France
- 10.30-10.45** **Phenotypic spectrum of hematological and visceral disease in type 3 Gaucher disease : Genotype correlations and response to Imiglucerase therapy in 380 Patients from the International Collaborative Gaucher Group (ICGG) Gaucher Registry.**
Pramod Mistry, USA
- 10.45-11.00** **Analysis of brains from a mouse model of neuronopathic Gaucher disease reveals progressive and localized pathology from birth.**
Ahad Rahim, UK
- 11.00-11.15** **The role of novel, non sphingolipid storage molecules in the pathogenesis of type 3c Gaucher disease.**
Jonathan Roos, UK
- 11.15-11.30** **Cell surface associated glycohydrolases in normal and Gaucher disease fibroblasts.**
Sandro Sonnino, Italy
- 11.30-11.45** **Long-term follow up of the Mainz cohort of Gaucher disease type 3 patients.**
Jörg Reinke, Germany
- 11.45-12.15** **Session 3: European Gaucher Alliance**
Chairs : Jeremy Manuel O.B.E. , UK ; Carla Hollak, The Netherlands
- Presentation.**
Tanya Collin-Histe ,UK, Pascal Niemeyer, Germany
- 12.15-13.15** **Lunch**

- 13.15-14.15** **Poster tour 1 : Posters 1-22**
(presenting authors should be present at poster).
Atul Mehta, UK ; Eugen Mengel, Germany : Posters 1-11
Marc Berger, France ; Maciej Machaczka, Sweden : Posters 12-22
- 14.15-15.15** **Session 4: Immunology**
Chairs : Hanna Rosenbaum, Israel ; Marc Berger, France
- 14.15-14.30** **Basis of immune dysregulation in Gaucher disease.**
Ozlem Goker Alpan, USA
- 14.30-14.45** **Fatal B-cell lymphoma in experimental Gaucher disease.**
Elena Pavlova, UK
- 14.45-15.00** **Severe impairment of regulatory T-cells and other numerical peripheral blood T-lymphocyte abnormalities in patients with Gaucher disease.**
Christos Sotiropoulos, Greece
- 15.00 -15.15** **Multilineage dysplasia of bone marrow is a frequent phenomenon in untreated Gaucher disease type 1.**
Monika Klimkowska, Sweden
- 15.15 – 16.30** **Session 5: Diagnosis and monitoring**
Chairs :Miguel Pocovi, Spain ; Atul Mehta, UK
- 15.15-15.35** **Preventing lysosomal storage diseases by preimplantation genetic diagnosis.**
Gheona Altarescu, Israel
- 15.35-15.45** **Transient elastography (TE) of spleen (Fibrospleen) and liver (Fibroscan) in patient with splenomegaly : A pilot study.**
Stephan vom Dahl, Germany
- 15.45-16.00** **Is measurement of liver elasticity and liver iron content of value in patients with Gaucher disease?**
Laura Van Dussen, The Netherlands
- 16.00- 16.15** **Fine needle aspiration versus trephine biopsy of bone marrow – comparison of utility in diagnostics of sporadic cases of Gaucher disease type 1–A cytohistological study.**
Maciej Machaczka, Sweden
- 16.15-16.30** **Molecular profiling of Gaucher disease by Fourier transform infrared spectroscopy.**
Serap Dokmeci, Turkey
- 16.30 -17.00** **Coffee Break**

- 17.00-18.30** **Session 6 : Meet the experts :** *Timothy Cox, UK ; Pramod Mistry, USA
Ari Zimran, Israel*
- Educational session**
**Dedicated for young physicians and new experts : Clinical, biological
and imaging aspects, treatment, around case presentations, Q and A.**
- 17.10-17.15** **Diagnosing Gaucher disease : An ongoing need for increased
awareness amongst haematologists.**
Derralyn Hugues, UK
- 17.15-17.30** **Mesenteric and mediastinal lymphadenopathy in egyptian children
with Gaucher disease : A study of 6 patients : Can enzyme
replacement therapy (ERT) play a role in improving their condition ?**
Magy Abdelwahab, Egypt
- 17.30-17.45** **Gaucher disease and malignancies : Diagnostic and therapeutic
challenges.**
Hanna Rosenbaum, Israel
- 17.45-18.30** **Case presentations.**
- 17.00-19.00** **Business meeting of the EWGGD (for EWGGD Members).**
- 20.00** **Congress dinner at the hotel**

Friday, June 29, 2012

- 08.30-09.00** **Invited Lecture**
β-Glycosphingolipids as immune modulators for oral immune therapy.
Ilan Yaron, Israel
- 09.00-10.00** **Session 7: Bone disease I**
Chairs : Derralyann Hughes ; UK, Argiris Simeonidis, Greece
- 09.00-09.15** **Long term bone mineral density response to enzyme replacement therapy in a retrospective pediatric cohort of Gaucher patients.**
Bruno Bembi, Italy
- 09.15-09.30** **Glucocerebrosidase deficiency in zebrafish leads to primary osteogenic defects.**
Enrico Moro, Italy
- 09.30-09.45** **Glucocerebrosidase deficiency in mesenchymal stem cells from a cohort of 10 Gaucher disease type 1 patients leads to abnormal osteogenesis.**
Nadia Belmatoug, France
- 09.45-10.00** **Taliglucerase alfa leads to favorable bone marrow responses in patients with type 1 Gaucher disease.**
Laura Van Dussen, The Netherlands
- 10.00-10.30** **Coffee break**
- 10.30-12.00** **Session 8: Bone disease II**
Chairs : Elena Lukina, Russia ; Carla Hollak, The Netherlands
- 10.30-11.00** **Invited lecture**
Pathophysiology of skeletal manifestations in type 1 Gaucher disease.
Pierre Lafforgue, France
- 11.00-11.15** **Abnormal hemorheological and adhesion properties of red blood cells in Gaucher disease.**
Cyril Mignot ; Yves Colin-Aronovicz, France
- 11.15-11.30** **Histological findings of femoral heads from patients with Gaucher disease treated with enzyme replacement.**
Ehud Lebel, Israel
- 11.30-11.45** **Bone parameters in adults with type 1 Gaucher disease treated with velaglucerase alfa in trial TKT025 and the extension study : Focus on the bone marrow burden scores over 7 years.**
Deborah Elstein, Israel
- 11.45-12.00** **Whole body MRI technique in early treated non-neuronopathic patients with enzyme replacement therapy at least eight years.**
Larissa Moos, Germany
- 12.00-13.00** **Lunch**

- 13.00-14.00** **Poster tour 2 : Poster 23-44**
(presenting authors should be present at poster).
Hanna Rosenbaum, Israel ; Jonathan Roos, UK : Posters 23-33
Helen Michelakakis, Greece ; Gheona Altarescu, Israel : Posters 34-44
- 14.00-15.00** **Session 9: Patient care**
Chairs: Liz Morris, UK ; Anat Oz, Israel
- 14.00-14.15** **Invited lecture**
The nurse experience.
Liz Morris, UK
- 14.15-14.30** **Invited lecture**
The patient experience.
Wout Timmerman, The Netherlands
- 14.30-14.45** **The unique role of the clinical trial nurse.**
Yehudit Chen Zion, Israel
- 14.45-15.00** **Design of a therapeutic patient education for Gaucher disease patients: The French experience.**
Christine Serratrice, France
- 15.00-15.30** **Coffee break**
- 15.30-16.30** **Session 10 : Novel and established therapies I**
Chairs: Maciej Machaczka, Sweden ; Ari Zimran, Israel
- 15.30-15.45** **A Phase 2 multicenter, open label, switch over trial to evaluate the safety and efficacy of Abcertin® (Imiglucerase) in patients with type 1 Gaucher disease previously treated with Imiglucerase.**
Han Wook Yoo, Korea
- 15.45-16.00** **ZAGAL Study : Long-term management and follow-up of use of Miglustat in type 1 Gaucher disease in Spain.**
Pilar Giraldo, Spain
- 16.00-16.15** **Eliglustat, an investigational oral therapy for Gaucher disease type 1 : Phase 2 results after 4 years of treatment.**
Elena Lukina, Russia
- 16.15-16.30** **Velaglucerase as therapy for Gaucher disease.**
Deborah Elstein, Israel
- 16.30-17.00** **Coffee break**

- 17.00-18.00** **Session 11 : Parkinson disease**
Chairs : Mia Horowitz, Israel ; Derek Burke, USA
- 17.00-17.15** **Incidence of Parkinson disease in obligate carrier relatives of patients with Gaucher disease : A single-center report.**
Deborah Elstein, Israel
- 17.15-17.30** **Gaucher disease : ERAD of mutant glucocerebrosidase variants in Parkinson disease.**
Inna Bendikov-Bar, Israel
- 17.30-17.45** **The E3 ligase itch regulates degradation of mutant glucocerebrosidase.**
Gali Maor, Israel
- 17.45-18.00** **Psychosines implicated in the pathogenesis of Gaucher disease alter actin dynamics and perturb intracellular lysosomal trafficking.**
Jonathan Roos, UK
- 21:00 Meeting point at the Congress Welcome Desk
21.30–23.45 Dinner on the Seine at Capitaine Fracasse

Saturday, June 30, 2012

- 08.30-10.00** **Session 12 : New treatment perspectives : Basics and politics**
Chairs: Helen Michelakakis, Greece ; Stephan vom Dahl, Germany
- 08.30-08.45** **Competition law and Gaucher and other orphan drug pricing.**
Hanna Hyry, UK
- 08.45-09.15** **Invited lecture**
Investigation of a novel approach to treat patients with Gaucher disease.
Roscoe Brady, USA
- 09.15-09.30** **Activation of the unfolded protein response in Gaucher disease.**
Mia Horowitz, Israel
- 09.30-09.45** **Exploring bicyclic derivatives of L-idonojirimycin as pharmacological chaperones for the treatment of Gaucher disease.**
Pilar Alfonso, Spain
- 09.45-10.00** **The pharmacological chaperone AT3375 alone and in combination with recombinant human acid β -glucosidase for Gaucher disease.**
Benjamin Elfrida, USA
- 10.00-10.30** **Coffee break**
- 10.30-11.50** **Session 13 : Established and novel therapies II**
Chairs : Pramod Mistry, USA ; Marieke Biegstraaten, The Netherlands
- 10.30-10.45** **Long-term clinical outcomes in type 1 Gaucher disease following 10 years of Imiglucerase treatment.**
Stephan vom Dahl, Germany
- 10.45-11.00** **Plant-cell-expressed recombinant glucocerebrosidase - Taliglucerase alfa as therapy for Gaucher disease in patient previously treated with Imiglucerase.**
Gregory Pastores, USA
- 11.00-11.15** **A multicenter, double-blind, randomized safety and efficacy study of two dose levels of Taliglucerase-alfa in pediatric subjects with Gaucher disease.**
Ari Zimran, Israel
- 11.15-11.30** **The French Gaucher disease registry: Clinical characteristics, complications and treatment of 562 patients.**
Jérôme Stirnemann, France
- 11.30-11.50** **Developing lentiviral vectors for gene therapy of type 1 Gaucher disease.**
Katherine Aitchison, UK
- 11.50-12.15** **Poster Prices**
Concluding remarks
Invitation to the next EWGGD meeting

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On the value of fundamental laboratory research: novel chemical tools and methods to study Gaucher disease pathophysiology and therapies.

Aerts JMFG.

Department of Medical Biochemistry, Academic Medical Center, UvA, Amsterdam

At the start of the talk, Professor Joseph Tager, one of the two founding fathers of the EWGGD who peacefully deceased last year, is commemorated. Joseph Tager was a stimulating mentor for many: his characteristic optimism and zeal to discover new things and to translate new insights to some clinical benefit has been contagious. His thoughts on the need for independent and objective applied biomedical research on Gaucher disease and in parallel the necessity of ongoing fundamental investigations, both in academic settings, will be addressed.

In some aspects, Gaucher disease is presently the best understood lysosomal storage disorder. The primary underlying biochemical cause is proven to be insufficient activity of the lysosomal acid beta-glucosidase (glucocerebrosidase; GBA1). Such intracellular condition causes tissue macrophages to transform in characteristic lipid-laden Gaucher cells. The consequences of deficient GBA1 activity in other cell types are still poorly known, probably contributing to some of the remaining enigmas in the pathophysiology of Gaucher disease. In the common non-neuronopathic type 1 variant, visceral Gaucher cells are the most prominent abnormality. Satisfactory clinical responses are observed with treatment of this disease variant by means of chronic enzyme supplementation of macrophages, for which nowadays multiple enzyme preparations are registered. Alternatively, small compounds are presently developed and studied for substrate reduction therapy and chaperone therapy of Gaucher disease. Applied research has very much dominated the Gaucher field in the last decade. The recent availability of mouse models and novel technologies has boosted a new phase of fundamental research. In this presentation attention will be focused to novel achievements in fundamental laboratory research on GBA1 and Gaucher disease in Amsterdam.

We designed a class of activity-based probes allowing specific and sensitive visualization of GBA1 *in vitro* and *in situ*. Using these probes, covalently attaching to the key nucleophile in the catalysis, the half-life of GBA1 can be determined in cultured cells and different tissues of mice. Visualization of enzyme labeled with fluorescent becomes possible following SDS-PAGE, by FACS or microscopy. It is also feasible to follow the fate of labeled therapeutic enzymes in cultured macrophages and mice. Expanding the approach, we have very recently developed another class of activity-based probes that besides GBA1 also label the non-lysosomal glucocerebrosidase GBA2. Evidence is presented that over-activity of GBA2 plays a role in neuropathology in Niemann-Pick Type C, reminiscent in some aspects to that in neuronopathic Gaucher disease. The therapeutic potential of hydrophobic iminosugars in this respect is addressed.

Regarding lipid storage following GBA1 deficiency, attention has been almost entirely focused on the lipid glucosylceramide. We have noted secondary, possibly adaptive, changes upon GBA1 inactivation in cells and mice, such as increases in gangliosides and the striking formation of glucosylsphingosine. The potential (patho)physiological implications of these will be discussed. Newly discovered surrogate disease markers, proteins as well as the lipid glucosylsphingosine, will be described and their possible merit in monitoring of disease prior and during therapies is evaluated.

Phenotypic spectrum of hematological and visceral disease in type 3 Gaucher disease: Genotype correlations and response to Imiglucerase therapy in 380 patients from the International Collaborative Gaucher Group (ICGG) Gaucher Registry.

Mistry PK¹, Kolodny EH², Tylki-Szymanska A³, Belmatoug N⁴, Cabello JF⁵, Vellodi A⁶, Cole JA⁷, El-Beshlawy A⁸, Grabowski G⁹.

¹Yale University School of Medicine, New Haven, CT, USA; ²New York University School of Medicine, New York, USA; ³Children's Memorial Health Institute, Warsaw, Poland; ⁴Centre de Référence des Maladies Lysosomales Assistance Publique-Hôpitaux de Paris Service de Médecine Interne Hôpital, Beaujon, France; ⁵Laboratorio de Genética y Enfermedades Metabólicas. INTA, Universidad de Chile, Santiago, Chile; ⁶Great Ormond Street Childrens' Hospital NHS Trust, London, UK; ⁷Biostatistics/Epidemiology, Genzyme, a Sanofi Company, Cambridge, MA, USA; ⁸Pediatric Hospital of Cairo University, Cairo, Egypt; ⁹Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Introduction: The neuronopathic Gaucher disease (GD) mutation L444P may be the most prevalent mutation across all ethnicities globally due to vulnerability of the *GBA* locus for gene conversion events. The full phenotypic spectrum, genotype distribution, and long-term outcomes with imiglucerase therapy among patients with Type 3 GD (GD3) are not known.

Aim: Define the phenotypic spectrum of hematologic and visceral disease, *GBA* genotype distribution and long-term outcomes with alglucerase/imiglucerase therapy in GD3 patients.

Methods: All patients enrolled in the International Collaborative Gaucher Group (ICGG) Gaucher Registry (*ClinicalTrials.gov* NCT00358943) as of October 2011 and diagnosed with GD3 were included in this analysis. Clinical parameters (hemoglobin level, platelet count, hepatic and splenic volumes, height Z-scores) were assessed at baseline and up to 5 years following initiation of therapy. Multivariate regressions were used to characterize baseline status as a function of covariates. We also assessed long-term survival in GD3 by Kaplan-Meier analysis and cause of death.

Results: A total of 380 patients were identified; most were from the Middle East, Europe, and the US. The majority of patients were diagnosed and initiated treatment before 6 years of age. *GBA* gene analysis revealed that L444P and D409H represented 78% and 14% of disease alleles. At baseline, age was correlated with baseline hemoglobin level, platelet count, and height Z-score; correlations were less pronounced for liver and spleen volumes. The majority of patients exhibited a dramatic reversal of visceral and hematologic disease within the first year of treatment, with incremental reversal of disease parameters over the 5 years, as measured by hemoglobin levels, liver and spleen volumes, and height Z-score. Among patients who died (16%, 62/380) and had reported causes of death, the most frequently cited were progressive neurological disease, infections, cardiac complications, an malignancy.

Discussion: The vast majority of GD3 patients present in early childhood with devastating visceral and hematologic disease and growth failure. These life-threatening complications were stopped and rapidly reversed by alglucerase/imiglucerase treatment, with further improvement after ≤ 5 years of treatment. Enzyme replacement therapy with alglucerase/imiglucerase results in remarkable improvements for patients with GD3, a life-threatening disorder with significant associated mortality.

Analysis of brains from a mouse model of neuronopathic Gaucher disease reveals progressive and localized pathology from birth.

Rahim AA¹, Richard-Loendt A², Osellame L³, MS. Wong A⁴, Mukherji S¹, Duchen MR³, Brandner S², Cooper JD⁴, Waddington SN¹.

¹ Gene Transfer Technology Group, Institute for Women's Health, University College London, London, UK.

² Department of Neurodegenerative Disease, Institute of Neurology, University College London, London, UK

³ Department of Cell and Developmental Biology, University College London, London, UK.

⁴ Pediatric Storage Disease Laboratory, Institute of Psychiatry, King's College London, UK.

Introduction: Type II neuronopathic Gaucher disease is a lysosomal storage disorder caused by mutation within the *GBA1* gene. The result is an acute and rapidly progressive neurological disease that results in death by 2 years of age. Systemically administered enzyme replacement therapy is ineffective due to the impermeable blood-brain barrier and conventional medicine offers no therapeutic protocol. Alternative approaches have been suggested such as gene or stem cell therapy. However, a better understanding of disease pathology and the window of therapeutic opportunity are required, especially in such a rapidly progressive disease where substrate accumulation has been reported at birth in patients. **Aim:** To assess various markers of disease pathology in the brains of a mouse model of type II Gaucher disease. This will involve monitoring pathology from birth to death and a comparative study between wildtype, heterozygous and *GBA1* knockout mice.

Methods: Brains from post gestation day 1 (P1) (pre-symptomatic), P8 (pre-symptomatic) and P12 (post-symptomatic and just before death) were taken from wildtype, heterozygote and homozygous knockout mice. Furthermore, the body weight of mice was recorded prior to culling. The brains were fixed, sectioned and examined by immunohistology using a range of markers for pathology: microglia-mediated inflammation (CD68 and Iba-1), astrogliosis (GFAP), myelin (myelin basic protein), neurodegeneration (NeuN), synaptic patterns (synaptophysin) and apoptosis (cleaved-caspase 3). Measurements of cortical thickness and volume were recorded to examine for atrophy. Thresholding image analysis of discrete areas of the brains stained with markers for inflammation and astrogliosis were conducted to elucidate areas more susceptible to pathology. Stereology was conducted on Nissl stained brain sections to quantify neurodegeneration in specific regions as the disease progresses. We also examined primary neuronal and astrocyte cultures from the different genotypes for dysfunctions in mitochondria, mitophagy and autophagy.

Results: Basic hematoxylin and eosin staining revealed severe and widespread neurodegeneration and vacuolation in most areas of the brain, particularly the brain stem. Immunohistological examination of microglia-mediated inflammation revealed extensive staining, most significant in the brain stem but also the ventral posterolateral/ventral posteromedial nucleus (VPL/VPM) and layer V pyramidal neurons of the cerebral cortex in knockout mice compared to heterozygous and wildtype controls. This was evident at birth in the brain stem and progresses to other areas rapidly as seen at P8 and P12. Astrogliosis was also prominent in the brain stem and significantly so at birth in knockout mice when compared to control heterozygotes and wildtype mice. As the disease progresses, this spreads to other regions, again most significantly in the VPL/VPM and layer V of the cortex. This provides a temporal and spatial correlation between inflammation and astrogliosis. Immunohistology and stereology both revealed significant and progressive neuronal loss in these areas of interest. We detect a widespread and significant increase in apoptotic nuclei, particularly in the brain stem, substantia nigra and hippocampus (the stem cell region of the dentate gyrus) when compared to control brains. Interestingly, despite significant pathology, we do not measure any significant difference in cortical thickness or volume and we find no evidence of disruption of myelinated fibres and synaptic patterns appear to be normal. Examination of mitochondrial function in primary cultures taken from P1 mice revealed significant morphological aberrations and low membrane potential that is not due to respiratory chain substrate supply.

Discussion: Disease pathology in this mouse model of type II Gaucher disease is present at birth and progresses rapidly through most areas of the brain. However, this is most significant in discrete regions such as the brain stem, VPL/VPM, cortex and hippocampus. Furthermore, dysfunction in mitochondria, mitophagy and autophagy are present in neuronal and astrocyte primary cultures from P1 mice. Taken together, these data suggest that particular regions of the brain are more susceptible to pathology than others and that the window for therapeutic intervention is very narrow. An early neonatal therapy may already be too late, leading to the suggestion that an in utero approach may be required.

The role of novel, non-sphingolipid storage molecules in the pathogenesis of Type IIIc Gaucher disease

Roos JCP, Stein PE, Cox TM.

Department of Medicine, University of Cambridge.

Introduction : Type IIIc is a rare variant of Gaucher disease with cardiac and ocular features reminiscent of some mucopolysaccharidoses. It is associated uniquely with a specific mutation in glucocerebrosidase at position D409H.

Aim : To determine if this phenotype might result from a preferential loss of a secondary activity of glucocerebrosidase affected by position 409, and consequently be characterized by the accumulation of a novel storage compound, possibly akin to the glycosaminoglycans known to cause MPS disease.

Methods : We used structural modelling, enzyme assays of mutant recombinant enzyme, culture of Type IIIc patient fibroblasts and analysis of Type IIIc urine for lipids and other compounds to investigate the pathogenesis of this rare Gaucher variant.

Results : We found that as shown by others, β -glucocerebrosidase (recombinant and placental) have acid β -xylosidase activity between 2-5% of their acid- β -glucosidase activity using artificial substrates and with a similar pH optimum. The D409H mutant enzyme showed no preferential loss of β -xylosidase activity; however recombinant D409H β -glucocerebrosidase accumulates less rapidly than wild type in culture suggesting reduced stability. Structural interpretation suggests that the aspartate at position 409 forms a salt bridge with serine 97, stabilizing the α helix; β -glucocerebrosidase is structurally related to enzymes involved in xyloside, xylan and arabinofuranoside cleavage. Consequently, we showed that the Gaucher enzyme has activity against α -L-arabinofuranoside, glucopyranosiduronic acid and β -D-fucoside. However imiglucerase and alglucerase differ in that the former is not recognized by a monoclonal antibody raised against the natural enzyme, suggesting that a single point mutation near the end of the molecule and perhaps vicinal to D409H can affect 3-D structure. We confirmed that mutation at position 495 does not affect catalytic specificity. Alglucerase may have contained other proteins obscured by the presence of albumin on electrophoresis and that these may include enzymes capable of cleaving α -D-mannopyranoside, N-acetyl β -D-glucosaminide and 2-acetamido-2-deoxy β -D -glucopyranoside. Finally, we demonstrate that oxalate accumulates excessively in at least 1 patient with advanced Type IIIc disease and is higher than average in younger patients with less advanced disease.

Discussion : We investigated the role of alternative enzymatic activities in the Gaucher enzyme in causing Type IIIc disease and the putative structural effect of D409H. We found a possible hyperoxaluria in Type IIIc disease and propose a novel mechanism by which the enzyme deficiency might account for oxalate excess.

Cell surface associated glycohydrolases in normal and Gaucher disease fibroblasts.

Aureli M, Bassi R, Loberto N, Regis S°, Prinetti A, Chigorno V, Aerts JM*, Boot RG*, Filocamo M°, Sonnino S.

Department of Medical Biotechnology and Translational Medicine, University of Milan;

°"Diagnosi Pre-Postnatale Malattie Metaboliche" Laboratory, G. Gaslini Institute, Genova;

*Department of Medical Biochemistry, Academic Medical Center, University of Amsterdam,

Sphingolipid (SL) metabolic processes occur mainly inside cells, but many studies have identified enzymes associated with the PM that are capable of modifying the SL head group and the membrane properties. In addition, these enzymes interact with the glycolipids that are part of the neighboring cell plasma membranes, are involved in cell-cell cross-talk and finely regulate glycosphingolipid (GSL)-protein interactions. β -glucocerebrosidase, like other glycohydrolases involved in glycosphingolipid (GSL) metabolism, is present in both plasma membrane (PM) and intracellular fractions.

Many of the molecular defects that underlie SL storage diseases (SLSDs) are known. However, the clinical phenotypes observed in SLSDs are not a simple consequence of the accumulation of a specific SL in lysosomes, but rather result from complex mechanisms (both direct and indirect) involving general SL intracellular trafficking and turnover.

In the present study, we evaluated the activities of CBE-sensitive β -glucosidase (GBA1) and AMP-DNM-sensitive β -glucosidase (GBA2), together with other glycohydrolases, in total cell lysates and PM of human fibroblast cell lines from control (normal) subjects and from patients with GD clinical types 1, 2, and 3.

GBA1 activities in both total lysate and PM of GD fibroblasts were low, and their relative percentages were similar to those of control cells. In contrast, GBA2 activities were higher in GD cells than in control cells, and the degree of increase differed among the three GD types. The increase of GBA2 enzyme activity was correlated with increased expression of GBA2 protein as evaluated by QRT-PCR. Activities of β -galactosidase and β -hexosaminidase in PM were significantly higher for GD cells than for control cells and also showed significant differences among the three GD types, suggesting the occurrence of cross-talk among the enzymes involved in GSL metabolism.

Our findings indicate that the profiles of glycohydrolase activities in PM may provide a valuable tool to refine the classification of GD into distinct clinical types.

Long term follow up of the Mainz cohort of GD type 3 patients.

Arndt J, Reinke J, Heidrich A, Brixius-Huth M, Mengel E.

Villa Metabolica, Center of Pediatric and Adolescence Medicine, MC JGU Mainz, Germany.

Introduction: During the last 15 years ERT had changed the natural history of GD type 3. In old text books an average age of death was reported at the age of 12 years. In the most cases non-neurological complications of GD had limited life time, however nowadays non-neurological complication may be prevented with ERT.

Patient cohort: 22 patients in the mean age of 17 years (SD +/- 8 years) were followed about in mean 9 years (+/- 7 years). The patients were followed by careful neurological examinations and die mSST score published by Davies et al. 2011. GBA Mutation analyses was performed in all patients, but in one patient GBA gene is still under investigation.

Discussions: 2 patients deceased due to initiation of ERT behind time. In the others survival is marked longer than in historical cohorts. Progression rate in the mSST score is less than 1 point per 2 years in patients without myoclonic epilepsy. 2 patients had progressive myoclonus epilepsy. All patients had only a small group of point mutations. Patients not compound-heterocygous or homocygous for D409H and L444P tend to have a higher progression rate with cognitive decline and loss of motor function. 3 patients with adult onset had epilepsy as prominent neurological sign.

Conclusion: As neurological progression is low in the most patients with GD3, they have meaningful benefit from ERT, when timely initiated. The most evident loss of scoring points takes place in the preschool age, which is important for an hypothetical therapeutic window. Prognostic factors for bad outcome are late diagnosis, myoclonic epilepsy, mutations others than D409H and L444P and splenectomy.



What is Gaucher Disease?



Philippe Charles Ernest Gaucher (1854-1918)

Gaucher Disease is an autosomal recessive disease and the most common lysosomal Storage Disorder with an incidence of about 1 in 100,000 live births. It is caused by deficiency of a specific enzyme (glucocerebrosidase) in the body, caused by a genetic mutation received from both parents. This leads to an accumulation of the enzyme's substrate, glucocerebroside, which results in it being stored in the spleen, liver, kidneys, lungs, brain and bone marrow. Each disease course can be variable, ranging from no outward symptoms to severe disability and death.

Symptoms may include enlarged spleen and liver, liver malfunction, skeletal disorders and bone lesions that may be painful, neurologic complications, swelling of lymph nodes and (occasionally) adjacent joints, distended abdomen, a brownish tint to the skin, anemia, low blood platelets and yellow fatty deposits on the sclera. Persons affected most seriously may also be more susceptible to infection. Despite the fact that Gaucher Disease consists of a phenotype, with varying degrees of severity, it has been sub-divided in three subtypes according to the presence or absence of neurological involvement.

Treatment of Gaucher disease

Two different types of therapies for Gaucher patients are currently approved:

Enzyme Replacement Therapy (ERT) and Substrate Reduction Therapy (SRT). Other treatment options, e.g. gene therapy, small molecule and chaperone therapy, are in the development phase. There are also several clinical trials currently being undertaken in patients with Gaucher disease.

Enzyme replacement therapy – with ERT, the missing or deficient enzyme (glucocerebrosidase) is replaced with a functional enzyme. This enzyme takes over the function of patient's deficient enzyme and breaks down the accumulated enzyme's substrate (glucocerebroside). Enzyme is administered intravenously at regular intervals throughout an individual's lifetime.

Substrate reduction therapy – in the SRT, the formation of the enzyme substrate (glucocerebroside) is inhibited by a small molecule. As a result, cells have less substrate and its accumulation is stopped. Small molecule is administered orally, in a tablet form.

European Gaucher Alliance (EGA)

The European Gaucher Alliance (EGA) is an incorporated company which was registered in England in 2008 with an elected a board of directors. Its membership is made up of National groups representing Gaucher Patients and it currently has 28 Full members and 3 Associate members (non voting membership is available to National Gaucher Groups outside Europe).

Representatives of European Gaucher patient groups first met in Trieste in 1994 at a meeting of the European Working Group on Gaucher Disease (EWGGD) and have come together in separate sessions at subsequent meetings of the EWGGD. The Aims of the EGA are set out below but a prime objective is to achieve access to expert treatment for Gaucher patients wherever they are in the world.

The EGA is particularly proud to have been instrumental in the establishment by the Genzyme Corporation of the European Cerezyme Access Program (ECAP) which has now merged with others to become the International Cerezyme Access Program which provides Humanitarian aid to Gaucher patients and has resulted in 120 patients from 14 countries accessing treatment which otherwise would have not been available to them.



The Aims of the EGA are:

- To collect information on the latest developments in the understanding, management and treatment of Gaucher Disease and to disseminate such information to all parties who have an interest in Gaucher Disease and other similar disorders.
- To provide information support guidance and encouragement to groups of individuals representing Gaucher patients throughout Europe and elsewhere in the world.
- To represent the interests of Gaucher patients to European and International organisations and bodies and to ensure that the voice of the Gaucher Patient is heard at all times.
- To encourage and promote scientific and medical research into Gaucher Disease and improved therapeutic approaches and to seek to ensure all such research recognises the centrality of the Gaucher Patient.
- To work with the medical and scientific community to define priorities in the understanding of Gaucher Disease its management and treatment.
- To work with, facilitate, support and encourage the activities of the European Working Group on Gaucher Disease (EWGGD) and other organisations or working groups with similar objectives.
- To be a forum to address ethical issues arising from the study of Gaucher Disease its management and treatment.
- To ensure that appropriate treatment is available to all patients with Gaucher Disease who require treatment regardless of race, creed, colour ethnic origin or national or religious background.

Achievements since formation:

- The EGA was formally Launched at the European Parliament under the sponsorship of Patron Peter Liese MEP in February 2009 when its board met with a number of MEPS and representatives of the European Commission.
- The EGA played a pivotal role with the EWGGD in the management the global Cerezyme crisis instigating and participating in meetings with the key European clinicians and industry and in the establishment of an emergency pool of enzyme to be made available to patients in Europe where treatment may otherwise not being available
- EGA members have contributed to papers in learned journals on the supply issues and also on the need for a centralised disease registry.
- EGA Members together with the EWGGD have participated in meetings with the European Parliament and three European Medicine Agency and engaged with Eurordis and other European bodies to seek to further the aim of the establishment of a European Gaucher Registry.
- The EGA together with the EWGGD and the US National Gaucher Foundation (NGF) called and hosted a meeting of the 5 leading pharmaceutical companies active in the field of Gaucher Disease to seek to develop a pathway to establish a Global Humanitarian program for Gaucher patients resulting the establishment of pilot studies for aid to India and Pakistan.
- The EGA has published and distributed the proceedings of the EWGGD meetings held in Budapest 2008 and Cologne 2010 and intends to do the same in June 2012 for the EWGGD to be held in Paris.
- EGA board members have worked with the Niemann-Pick European Patient Organisation and the Batters Disease Patient Organisation on the Establishment of pan European Patient Groups.
- A dedicated EGA website was launched in 2011.
- The EGA has been accredited by the European Medicines Agency as a recognised patient Organisation.

Meeting Plans for 2012:

- **EGA Meeting** – A full EGA meeting of its members associate members and prospective members will be held in Paris in June 2012 ahead of the EWGGD meeting at which members will participate
- **Go with Gaucher – taking forward the next generation** – this will take place in November 2012 and it aims are to bring together young Gaucher patients to work together on their common needs and aspirations and to encourage them to take active roles in their National Associations so they become the Gaucher leaders of tomorrow.

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Basis of immune dysregulation in Gaucher disease.

Martin C¹, Taber T¹, Unutmaz D², Goker-Alpan Ozlem¹.

¹ O&O Alpan LLC, ² NYU Langone Medical Center Dept. of Microbiology.

Introduction: Gaucher disease (GD), the inherited deficiency of glucocerebrosidase, is the most common Lysosomal Storage disorder. Activated lipid engorged macrophages are the hallmark for Gaucher disease (GD). Although the mechanisms for macrophage activation in GD is unclear, there is clinical evidence of immune dysregulation, and a non-specific inflammatory response, poor wound healing, frequent infections and predisposition to cancers are frequently encountered in patients with GD.

Aim: The objective of this study is to explore immune dysregulation in patients with Gaucher disease.

Methods: We performed immune profiling analysis in PBMC from GD patients (4M: 10F), (age range 34-56), and compared to healthy controls under an IRB approved protocol.

Results: We found the proportion CD14⁺ monocytes, CD4⁺ lymphoid dendritic cells and CD1d-restricted NKT cells were greatly reduced in GD patients ($p < 0.05$). In addition, while the total NK cell numbers were reduced, the proportion of NK cells displayed more activated/differentiated phenotype. There was also a profound decrease in naïve T cells.

Discussion: Clinically, these findings not only reflected disease severity but also correlated with response to therapy. Elucidating the link between immunological dysregulation in Gaucher disease will not only provide insights into novel functions of this lysosomal enzyme, but also further our understanding of the pathways that establish the cross talk between immune cells.

Fatal B-cell lymphoma in experimental Gaucher disease.

Pavlova EV¹, Wang SZ¹, Hook E², Dekker N³, Karlsson S⁴, Aerts JMFG³, Cox TM¹.

¹Department of Medicine, University of Cambridge, Cambridge UK

²Department of Histopathology, Addenbrooke's Hospital, Cambridge UK

³Academic Medical Center, Amsterdam, the Netherlands

⁴Lund Stem Cell Center, Lund University Hospital, Sweden.

Introduction : Multiple myeloma and B-cell lymphoma are leading causes of death in Gaucher disease (1) but the nature of the stimulus driving the often noted clonal expansion of immunoglobulin-secreting B cells and cognate lymphoid malignancy is unknown.

Aim : To determine if there is an increased frequency of these tumours in an authentic mouse model of chronic Gaucher disease.

Methods : Mice with inducible GBA1 deficiency in haematopoietic cells [Gba^{tm1Karl/tm1Karl}Tg(Mx1-cre)1Cgn/0 (MGI:3687965; MGI:2176073) were kindly provided by S. Karlsson (2). The colony was established by mating Gba^{tm1Karl/tm1Karl} with Gba^{tm1Karl/+}Tg(Mx1-cre)1Cgn/0 mice; and all offspring received parenteral inducer, (poly[I:C]). Animals of genotype Gba^{tm1Karl/tm1Karl}Tg(Mx1-cre)1Cgn/0 are termed GD mice; induced mice with the Gba^{tm1Karl/tm1Karl} and Gba^{tm1Karl/+} genotypes served as age-matched controls. Several mice became moribund ($\geq 20\%$ loss of maximum adult body mass) and obvious tumours were identified at post-mortem. Immuno-reactive pan B cell (B220), T cell (CD3) and macrophage (Mac-3) antigens were sought in random spleen, liver, lymph node or tumour samples. Plasma glucosylsphingosine and glucosylceramide concentrations were measured by mass spectrometry.

Results : These fatal tumours were diffuse B-cell lymphomas (10 out of 57 (17%) GD mice aged 0-24 months compared with 2 in 27 controls, trend X^2 df=1; p=0.05); unexpectedly, most mice with overt lymphoma had absent or few Gaucher cells but local inflammatory macrophages were present. Plasma glucosylsphingosine was greatly elevated in plasma of the GD mice (n=39) (median 57.9 nM; range 19.8–159); controls (n=29) (median 0.56nM; range 0.04–1.38) p <0.0001. In contrast, plasma β -glucosylceramide was not significantly different between the GD (median 3.70 μ M; 2.02 – 8.5) and control mice (median 5.13 μ M; range 0.74-9.6) but β -glucosylceramide was elevated as expected in liver and spleen tissue.

Discussion : Our strain of inducible GD mice has a high frequency of lethal B-cell malignancies: it serves as a tractable model to investigate the putative role of bioactive sphingolipids in the control of B-cell proliferation and in the pathogenesis of human lymphoid cancers associated with Gaucher disease.

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Severe impairment of regulatory T-cells and other numerical peripheral blood T-lymphocyte abnormalities in patients with Gaucher disease.

Sotiropoulos C¹, Theodorou G¹, Repa K², Marinakis T³, Karkalousos P², Kourakli A¹, Solomou E¹, Karakantza M¹, Symeonidis A¹.

¹Hematology Division, Dept of Int. Medicine, University of Patras Medical School, ²Polykliniki Hospital of Athens, ³Dept of Hematology, G.Gennimatas General Hospital of Athens, ⁴First Dept of Internal Medicine, Laikon Hospital of Athens, Greece.

Patients with Gaucher Disease (GD) manifest clinical and laboratory findings of a chronic inflammatory reaction, whose pathogenesis is obscure. We have previously shown that these patients exhibit decreased numbers of peripheral blood lymphocytes and of CD3+ and CD4+ T-cells, as well as increased CD8+CD45RO+ and CD8+HLA-DR+ T-lymphocytes. We investigated the frequencies of regulatory T-cells (T-regs), of Natural Killer (NK) cells and of autoreactive CD5+CD20+ B-lymphocytes in the same cohort of patients.

A 4-color whole blood flow cytometry was used for the evaluation of lymphocyte subpopulations, during the routine work-up of 25 patients with type I GD, after obtaining informed consent. T-regs were evaluated as the fraction of CD4+CD25^{high}+FOXP3+ cells. Results were compared with those of 22 sex-and age-matched controls. All estimations were performed in the absence of any infection or other inflammatory condition.

Again, patients with GD exhibited significantly decreased total lymphocytes ($x \pm SD$: 1724 ± 585 vs $2075 \pm 366/\mu\text{l}$, $p=0.030$), CD3+ lymphocytes (1178 ± 364 vs $1455 \pm 173/\mu\text{l}$, $p=0.005$) and CD3+/CD4+helper T-lymphocyte count ($40.2 \pm 7.8\%$ vs $45.1 \pm 5.7\%$, $p=0.028$ or 677 ± 226 vs $938 \pm 185/\mu\text{l}$, $p<0.001$). CD3+CD8+ suppressor T-cells were not significantly different. Patients with GD exhibited also increased proportions of activated CD4+CD25+ (2.68 ± 0.93 vs $2.02 \pm 0.90\%$, $p=0.018$), CD4+HLA-DR+ (2.74 ± 1.74 vs $1.10 \pm 0.48\%$, $p=0.0001$) and CD8+HLA-DR+ lymphocytes (1.01 ± 0.49 vs $0.48 \pm 0.19\%$, $p<0.0001$). CD8+CD25+ lymphocytes were not increased. Moreover, patients with GD had significantly lower CD4+CD25^{high}FOXP3+ T-regs (1.24 ± 0.52 vs $2.12 \pm 0.58\%$, $p<0.0001$). FOXP3+ cells were significantly lower among CD4+CD25^{high}+ cells (77.4 ± 16.2 vs $85.6 \pm 15.9\%$, $p=0.004$) and among CD4+CD25^{medium}+ cells (62.3 ± 16.5 vs $81.9 \pm 6.9\%$, $p<0.0001$). All NK-cell subpopulations (CD3+CD56+, CD3+CD56+CD57+, CD3-CD56^{dim}+, CD3-CD56^{dim}+CD16+, CD3-CD56^{high}+, and CD56^{high}+CD16+ cells) did not differ significantly between patients and controls. Finally both, the percentage and absolute number of CD5+/CD20+ autoreactive B-lymphocytes were not significantly different between patients and controls (3.53 ± 0.78 vs $1.94 \pm 0.37\%$, $p=0.087$ and 60 ± 18 vs $47 \pm 14/\mu\text{l}$, p : n.s.).

Our results demonstrate that patients with GD have a significant numerical impairment of T-helper lymphocytes and a constitutive activation of both, CD4+ and CD8+ cells, associated with a significant decrease of T-regs, although the autoreactive B-lymphocytes are not always found increased. These findings may explain the chronic inflammatory reaction and the increased incidence of lymphoid malignancies, which has been reported among G.

Multilineage dysplasia of bone marrow is a frequent phenomenon in untreated Gaucher disease type 1.

Klimkowska M¹, Kleinotiene G², Galazka K³, Jurczak W⁴, Mickys U⁵, Pileckyte R⁶, Bulanda A¹, Rageliene L², Hägglund H⁷, Tylki-Szymanska A⁸, Machaczka M⁷.

¹Department of Clinical Pathology and Cytology, Karolinska University Hospital, Stockholm, Sweden; ²Centre of Hematology and Oncology, Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Clinics, Vilnius, Lithuania, ³Department of Pathology and ⁴Department of Hematology, Collegium Medicum of the Jagiellonian University, Cracow, Poland; ⁵National Centre of Pathology, Vilnius University, Hospital Santariskiu Clinics affiliate, Vilnius, Lithuania, ⁶Department of Hematology, Oncology and Transfusion Medicine Center, Vilnius University Hospital Santariskiu Clinics, Vilnius, Lithuania, ⁷Department of Medicine at Huddinge, Karolinska Institutet, Stockholm, Sweden; ⁸Children's Memorial Health Institute, Department of Metabolic Diseases, Warszawa, Poland.

Introduction: Dysplasia is a morphological hallmark of disturbed cell or tissue growth in reactive conditions or neoplasia. Myelodysplastic syndromes (MDS) are a group of malignant diseases with clonal proliferation of abnormal bone marrow (BM) cells, with peripheral cytopenias and increased risk of progression to acute myeloid leukemia (AML). Patients with Gaucher disease (GD) often present initially with cytopenias, therefore myelodysplasia should be considered as a differential diagnosis. Coincidence of GD1 and MDS or AML was described by some authors but presence of dysplastic features was not extensively studied in GD1

Aim: To retrospectively analyze the presence of dysplastic features in bone marrow specimens from patients with GD1.

Material and methods: Bone marrow specimens obtained for diagnostic purposes from 17 untreated patients (pts) with GD1 from the three European countries (Sweden, Lithuania, and Poland), were retrieved and reassessed by a hematopathologist blind to clinical data. The specimens from 15 patients (7 women, 8 men) were eligible for an objective cytohistological assessment; bone marrow smears (BM-S) from 2 pts were extremely diluted with peripheral blood impeding BM cell assessment. The median age of these 15 pts at the time of BM sampling was 56 years (range 21–86 years), four pts (27%) were splenectomized. All but two pts carried at least one allele with a N370S (c.1226A>G) mutation in the *GBA1* gene. At the time of BM sampling, only one patient had no cytopenia in the peripheral blood, 7 pts had unilineage cytopenia, 5 pts had bicytopenia, and 2 pts had pancytopenia. Two pts had vitamin B12 deficiency. BM cellularity and GC burden were assessed in histological material. Two routinely stained (hematoxylin-eosin or May-Grünwald-Giemza) BM smears per patient and histological samples were analyzed for dysplastic features in three hematopoietic lineages according to the WHO classification criteria and methodology described by Verburgh et al, with a 10% threshold level for significant dysplasia.

Results: Significant dysplastic changes were as follows: in erythropoiesis - all pts, in myelopoiesis – 12 pts, in thrombopoiesis – 14 pts. Trilineage dysplasia was present in 11/15 pts, bilineage aberrations were found in 2 pts; two of the remaining pts had bilineage dysplasia plus equivocal myeloid picture. Discrepancies between BM and PB status (dysplasia in BM but no cytopenia in PB or vice versa) were noted for 7 pts in red cell series, for 3 pts in thrombopoiesis and for 12 pts in white cells. Of the two pancytopenic pts, one had bilineage and one had trilineage dysplasia. One patient with vit. B12 deficiency was bicytopenic and had trilineage dysplasia, whereas the other one had only a thrombocytopenia, with bilineage dysplasia and equivocal findings in myelopoiesis. Most pts had hypercellular BM (>50%, 11/15 pts) but only a few had a high GD burden (>40%). Four splenectomized patients had significant dysplasia in MGKs, and three of them had overt thrombocytopenia.

Discussion: The study suggests that significant dysplasia can be found in most GD1 patients, even in those without overt cytopenias or after splenectomy. Maturation of hematopoietic cells in BM can be disturbed by GC infiltrates by means of cytokine interactions, aberrant blood supply or advanced local fibrosis. The potential clonal nature of the observed abnormalities in hematopoietic cells in patients with GD remains to be investigated. Studies of patients who develop true MDS or AML in the course of GD can be of great value.

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Preventing lysosomal storage diseases by preimplantation genetic diagnosis.

Altarescu G^{1#}, Beerli R¹, Eldar-Geva T^{2#}, Elstein D³, Zimran A^{3#}, Margalioth EJ^{2#}, Levy-Lahad E^{1#}, Renbaum P¹.

Medical Genetics Institute¹, "ZOHAR" PGD Lab, Medical Genetics Institute²; IVF Unit; Gaucher Clinic³, Shaare Zedek Medical Center; affiliated with the Hebrew University School of Medicine[#], Jerusalem, Israel.

Background : Preimplantation genetic diagnosis (PGD) allows birth of unaffected children for couples at risk for a genetic disorder. We present the strategy and outcome of PGD for Gaucher disease (GD).

Material and Methods : We developed a fluorescent multiplex single cell PCR protocol that included familial mutation and at least six informative markers surrounding the mutation. Mechanical biopsy technique was performed and either polar bodies one and two or one single-cell (blastomere) of a six-cell embryo was analyzed.

Results : Analysis for non-neuronopathic GD was performed in four couples (one where both partners were also carriers of mutations in the Tay-Sachs disease HEXA gene) in which the predicted offspring phenotype was severe (based on parental glucocerebrosidase mutations: IVS2+1 and IVS12+1; 84GG and R359Q; 84GG and R496H; N370S homozygous affected partner and a 84GG carrier). Thirteen PGD cycles were performed by blastomere biopsy in these couples and 6 healthy children were born; a pregnancy rate/embryo transfer rate for these couples in whom there was no fertility problems was 46.2% compared to the overall pregnancy rate/embryo transfer among all couples with lysosomal disease performed in our unit (Fabry disease, Hunter syndrome, and Tay-Sachs disease) which was 38%.

Conclusion: PGD for Gaucher disease is a tenable option for couples for whom the predicted phenotype of affected offspring would be severe. It is a safe and effective method to prevent birth of affected children.

Transient elastography (TE) of spleen (Fibrospleen) and liver (Fibroscan) in patients with splenomegaly: a pilot study

Steinhof V, Seimel M, vom Dahl S.

Dept. of Internal Medicine/Gastroenterology, St. Franziskus-Hospital, Academic Teaching Hospital, University of Cologne, Schoensteinstrasse 63, 50825 Cologne, Germany

Introduction: Determination of liver stiffness by transient elastography (TE) with Fibroscan, a non-invasive ultrasound-like technique, is an established means of determining the degree of liver fibrosis in chronic hepatitis C and the transience from high fibrosis to cirrhosis in all chronic liver diseases. It is currently not known whether spleen elastography is feasible and whether fibrotic processes in the spleen can accurately be determined by this technique. Principally, splenomegaly can be caused by a) vascular changes, b) hyperplasia of red or white spleen pulp, c) storage material, d) infiltration by tumour cells, e) fibrosis, or f) a combination of these factors. Some Gaucher patients' splenomegaly remains resistant to therapy and it is thought that this is due to irreversible fibrosis of the spleen.

Aim: The purpose of the study was to clarify whether Fibrospleen in splenomegalic patients is feasible and whether splenomegalic patients principally exhibit increased spleen stiffness. Further the relation between liver and spleen stiffness and between spleen stiffness and spleen size was investigated.

Methods: Liver and spleen stiffness were determined by transient elastography (Fibroscan, Echosens™, Paris, France). Patient cohorts to be investigated were: healthy controls without splenomegaly (n=18), patients with GD type 1 (n=25, 7 splenectomized, all treated with either imiglucerase or velaglucerase), patients with compensated liver cirrhosis of different origins (n=29, >Child A), patients with splenomegaly of different origins (n=13, causes: HIV, NASH, mononucleosis, AML, NHL, others). In total, 85 patients were investigated (Tab.1). Transient elastography of liver was determined by standard techniques (TE medium probe (Echosens™, Paris, France), back supine position, laterally positioned TE probe, breathhold, ≥10 successful measurements). Determination of spleen stiffness was performed with the same TE probe in right lateral supine position, breathhold, ≥7 successful measurements).

Results: In Fibroscan measurements, >95 % of TE measurements were successful, whereas spleen TE was technically successful in >85 % of patients. The mean liver stiffness of cirrhotics was massively increased to $43,2 \pm 4,4$ kPa (n=29). In this group, spleen stiffness was significantly increased to $50,9 \pm 3,8$ kPa (n=29). Spleen size and stiffness did not exhibit a clear correlation.

Table 1: TE measurements (in kPa) in liver and spleen in different patient cohorts: P: significantly different from controls, n.s. not significant.

	Controls	Gaucher	Splenomegaly, other causes	Liver cirrhosis
Liver				
No.	18	25	13	29
Stiffness (kPa)	6,0	6,3	6,0	43,2
SD	2,0	2,0	1,3	23,7
SEM	0,5	0,4	0,4	4,4
P level		n.s.	n.s.	P<0.001
Spleen				
No.	18	16	13	29
Stiffness (kPa)	21,9	26,7	31,9	50,9
SD	8,7	8,0	19,8	20,2
SEM	2,1	2,0	5,5	3,8
P level		n.s.	P<0.05	P< 0.01

Discussion: Fibrospleen can accurately be determined in normal and splenomegalic patients, Accuracy of spleen elastography is less than Fibroscan of the liver, probably due to the smaller size of the spleen and its anatomical shape (curved, Margo crenatus). Highest TE values of spleen were obtained in patients with compensated liver cirrhosis. The TE values of either liver or spleen in stable Gaucher patients were not statistically different from healthy controls. Spleen stiffness seems to be determined primarily by vascular factors. Fibrospleen can not predict liver cirrhosis. Further studies have to show whether Fibrospleen can be used for monitoring diseases involving the spleen. Liver stiffness does not seem to differ from controls in the majority of Gaucher patients. Gaucher patients, whose spleen size and platelets are resistant to therapy, should be investigated by this technique. These patients were not present in the small Gaucher cohort examined in this pilot study.

Address of authors: St. Franziskus-Hospital Köln, Academic Teaching Hospital of the University of Cologne, Schönsteinstrasse 63, 50825 Köln, Telefon: +49-221-5591-1101, Telefax: +49-221-5591-1103, E-Mail: stephan.vomdahl@st-franziskus-koeln.de.

Grants: Parts of this study were supported by restricted research grants from Genzyme, Neu-Isenburg, Germany and the Deutsche Leber-Liga, Düsseldorf, Germany.

Is measurement of liver elasticity and liver iron content of value in patients with Gaucher disease?

Van Dussen L¹, Bohte AE², Akkerman EM², Nederveen AJ², Sinkus R³, Maas M², Aerts JMFG⁴, Jansen PLM⁵, Stoker J², Hollak CEM¹.

¹Department of Endocrinology and Metabolism, Academic Medical Center, University of Amsterdam, The Netherlands; ²Department of Radiology, Academic Medical Center, University of Amsterdam, The Netherlands; ³Centre de Recherche Biomédicale Bichat Beaujon (CRB3), INSERM U773, Beaujon Hospital, Clichy, France. ⁴Department of Medical Biochemistry, Academic Medical Center, University of Amsterdam, The Netherlands; ⁵Department of Gastroenterology and Hepatology, Academic Medical Center, University of Amsterdam, The Netherlands.

Introduction: Long term liver-related complications of Gaucher disease (GD) include fibrosis and increased risk to develop hepatocellular carcinoma (HCC). Splenectomy is a known risk factor for the development of liver pathology in GD. Accumulation of iron in Gaucher cells in the liver has been described and ferritin levels are usually elevated and serve as disease marker. Iron metabolism in Gaucher disease is not fully understood but iron storage may contribute to liver fibrosis. It is important to develop adequate diagnostic tools to identify at-risk GD patients.

Aim: We evaluated the potential usefulness of measuring fibrosis of the liver by MR elastography (MRE) and Fibroscan in patients with GD in relation to hepatic iron content as measured by MRI, disease markers and parameters of iron metabolism.

Methods: Twenty-one participants were included (17 male; mean age 49, range 24-70): 7 splenectomized (Sx) GD patients and 7 non-splenectomized (non-Sx) GD patients who were matched for age and gender with 7 healthy controls. All underwent liver iron concentration (LIC) measurement at 1.5T (Gandon, T2*), MRE at 3T and Fibroscan. Ferritin and HFE mutations (C282Y, H63D) were investigated in GD patients. Statistics were employed with non-parametric tests.

Results: MRE and fibroscan values were elevated in Sx GD patients: median MRE values were 2.52 kPa (range 2.37-2.64) for Sx GD and 1.64 kPa (range 1.31-2.12) for non-Sx GD ($p=0.006$). Median Fibroscan values were 8.8 kPa (range 3.5-16.0) for high-risk GD and 4.8 kPa (range 3.4-7.2) for low-risk GD ($p=0.025$). Non-Sx GD did not differ significantly from controls for MRE and Fibroscan. Despite differences in fibrosis, LIC did not differ significantly between the groups. LIC correlated with ferritin levels (spearman correlation coefficient 0.835, $p<0.001$). Eight GD patients had a HFE mutation: two C282Y heterozygotes, two H63D homozygotes and four H63D heterozygotes. Six of 7 Sx patients carried a HFE mutation versus 2 of 7 non-Sx patients.

Conclusion: MRE and Fibroscan can be used to monitor liver fibrosis and elasticity in patients with GD. LIC measurements correlated with ferritin levels but not with fibrosis. We hypothesize that altered iron metabolism may play a role in the pathology of Gaucher disease, although in this small group iron content was not directly correlated with fibrosis.

Fine needle aspiration versus trephine biopsy of bone marrow – comparison of utility and diagnostics of sporadic cases of Gaucher disease type 1 – A cytohistological study.

Machaczka M¹, Regenthal S², Bulanda A¹, Galazka K³, Kleinotiene G⁴, Wahlin BE¹, Hägglund H¹, Klimkowska M².

¹Department of Medicine and ²Department of Clinical Pathology and Cytology, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden; ³Department of Pathology, Collegium Medicum of the Jagiellonian University, Cracow, Poland; ⁴Centre of Hematology and Oncology, Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Clinics, Vilnius, Lithuania.

Introduction: Although bone marrow (BM) examination is generally no longer recommended for the sole purpose of Gaucher disease (GD) diagnosis, initial symptoms of sporadic GD type 1 (GD1) such as splenomegaly or thrombocytopenia, often lead to hematological diagnostics among non-Jewish patients. These routine diagnostic measures include fine needle aspiration and trephine biopsy of bone marrow for the cytological assessment of bone marrow smears (BM-S) and the histological evaluation of bone marrow trephine biopsies (BM-TB), respectively. Little is known about the comparison of accuracy of both methods in assessment of bone marrow involvement in GD, and further, about their utility in the diagnostics of sporadic cases of GD1.

Aim: To compare the accuracy of cytological versus histological assessment of BM in patients with GD1.

Methods: BM-S (May-Grünwald Giemsa stain) and BM-TB (hematoxylin-eosin stain), previously obtained from six non-Jewish, sporadic GD1 patients (2 female, 4 male) for diagnostic purposes, were retrospectively analyzed. The median age of the patients (pts) was 65 years (range 21–84 years), three of them (50%) were splenectomized. All but one patient (83%) carried at least one allele with a N370S (c.1226A>G) mutation. Differential counts of BM-S were estimated in the ×40 objective of Olympus BX 40 microscope; each patient sample consisted of 2 slides where 500 nucleated cells were counted on each slide. An assessment of the composition of BM-TB was carried out using digital photographs analyzed on a computer utilizing the Gimp 2 software. Photographic documentation of BM-S and BM-TB was made with a digital camera (Nikon DX m 1200F) in the Nikon Elipse E1000 microscope, using x40 objective for the BM-S and x2 respective x20 objectives for the BM-TB.

Results: The median number of GCs identified in BM-S in one patient was 4 (range 1–18), and the median percentage of GCs was 0.4% (range 0.1–1.8%). The absolute proportion of GCs in BM-TB ranged from 22–36% (median value 28%). The proportion of GCs to hematopoietic tissue ranged from 34–54% (median value 47%). In all 6 patients, the the median proportion of identified GCs to hematopoietic tissue were much larger in the BM-TB (range 34–54%) than in the BM-S (range 0.1–1.8%), and this difference was significant in Wilcoxon signed-rank test (P=0.028).

Discussion: Our results indicate that fine needle aspiration of BM is an unreliable diagnostic method to detect GCs in sporadic GD1 patients with unknown GD. The majority of the studied patients (4/6, 67%) had ≤6 GCs among 1,000 counted nucleated hematopoietic cells. Of note, routine differential counts of BM-S are usually performed on only 200 nucleated hematopoietic cells, and there is a serious risk of not diagnosing GD when using fine needle aspiration. GCs are often tightly packed within the regions engaged by GCs in BM and thus are difficult to aspirate, which can explain our finding. Additional problems with aspiration of GCs can result from an increased reticulin fibrosis of the BM. The sensitivity of trephine biopsy for detecting GCs in BM is high and, in our study, detected GCs burden was approx. 100 times higher compared with fine needle aspiration. However, trephine biopsy is not recommended for the sole diagnostic purpose in GD. We propose instead, that patients with unclear splenomegaly and/or thrombocytopenia, who display a negative result in BM-S assessment, proceed routinely to enzymatic diagnostics of GD (β-glucosidase and chitotriosidase activity assays).

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Molecular profiling of Gaucher disease by Fourier transform infrared spectroscopy.

İğci N¹, Sharafi P², Duygu Özel D¹, Yüce A³, Demiralp Ö⁴, Dökmeçi S(Emre)².

¹Ankara University Biotechnology Institute Proteomics Department. ²Hacettepe University Faculty of Medicine, Department of Medical Biology, ³Hacettepe University, Ihsan Dogramaci Children Hospital, Pediatric Gastroenterology, Hepatology and Nutrition Unit, ⁴Ataturk Training and Research Hospital, Department of Plastic and Reconstructive Surgery, Ankara, Turkey.

Introduction: Gaucher Disease (GD) is defined as an autosomal recessive disorder resulting from deficiency of glucocerebrosidase, also known as acid- β -glucosidase. Glucocerebrosidase is a lysosomal hydrolase responsible for the degradation of the natural glycosphingolipid, glucosylceramide, into ceramide and glucose. Deficiency of this enzyme results in the accumulation of undegraded glucosylceramide, almost exclusively in macrophages. Fourier Transform Infrared (FTIR) spectroscopy is a widely used and preferred method of infrared spectroscopy due to its speed and sensitivity. With FT-IR, complete molecular diversity of the samples can be studied comparatively with a knowledge of origins of the peaks (such as glycolipids, lipids, proteins etc.) as well as the amount of the particular materials can be determined. Also secondary structure ratios of proteins can be determined by analyzing the amide bands. These features provide invaluable information about functional and structural changes in cells underlying disease mechanisms.

Aim: The aim of this study is to achieve molecular characterization of biomolecules in GD in comparison with controls by using FTIR-ATR spectroscopy and cluster analysis.

Methods: Cultured skin fibroblast cells were used from 8 GD patients and healthy controls. Mid-infrared spectra were obtained using ATR cell by drying cells in PBS on the crystal using nitrogen gas. Spectral analyses were carried out by OPUS software and t-test was performed using SPSS software.

Results: 20 major absorption bands were assigned to various biomolecules in this study. As a result of the comparative evaluation, lipid and protein levels are seen to increased in GD. Bandwidth of CH₂ symmetric stretching of lipids is slightly decreased in GD, indicating the decrease in membrane fluidity. Additionally, we determined protein secondary structures and observed an increase in the ratio of antiparallel β -sheet and α -helix structures in GD, while β -sheet was decreased. Our cluster analysis yielded a classification within GD group as severe and moderate based on the lipid region. The band areas in this region show significant difference between severe and moderate groups.

Discussion: We report the first FT-IR spectrum of GD patient fibroblast cells in the mid-infrared region with their spectral assignments. Also this is the first FT-IR spectroscopic work aimed to determine molecular alterations in GD. Our results showed that FT-IR spectroscopy based methods may be useful for monitoring of GD patients as biomarkers and to reveal molecular mechanisms of GD.

Diagnosing Gaucher disease: an on-going need for increased awareness amongst haematologists.

Thomas AS, Mehta AB, Hughes DA.

Lysosomal Storage Disorders Unit, Royal Free Hospital, London & Department of Academic Haematology, University College London.

Introduction : Previous studies have reported a lack of awareness of Gaucher disease amongst physicians, including haematologists, contributing to diagnostic delays¹. The profile of Gaucher disease amongst the medical community in the UK has been raised, first by the approval of enzyme replacement therapy and new therapies and subsequently by the designation of specialist units.

Aim : To investigate if there is a trend to reduction in diagnostic delays in the modern era of Gaucher treatment and specialist services.

Method : a retrospective case note review of Gaucher patients attending the Royal Free Lysosomal Storage Disorders Unit. To ascertain:

1. Time from symptoms to diagnosis
2. Modality of diagnosis
3. Diagnosing speciality

Results : Most patients were referred after the availability of enzyme replacement. The commonest symptoms at presentation were related to splenomegaly and cytopenias. Few patients present primarily with bone pain, however, on initial evaluation over 50% of patients had bone disease severe enough to warrant enzyme replacement therapy. 10% of patients were diagnosed on family screening and 5% by community screening. Median time to diagnosis was 2 years (range 0.5-27 years). There was a trend to reduced diagnostic delay in recent years. The commonest diagnostic modality was bone marrow biopsy, consistent with the commonest diagnosing speciality being haematology. A few patients presented to hepatologists and orthopaedic surgeons. Outside the context of screening, enzyme assays were performed for confirmation rather than as the initial diagnostic test.

Discussion : The majority of patients present to haematologists with symptoms of bruising or related to splenomegaly. In contrast to patients with haematological malignancies, the history was prolonged with an absence of constitutional symptoms such as weight loss and fevers. Most patients were diagnosed as a result of reflex bone marrow testing to investigate abnormal blood tests. Despite the establishment of specialist centres, patients continue to experience long delays between the onset of symptoms and diagnosis. Although leucocyte enzyme assays have been available for over four decades, these are rarely utilised since tissue biopsies are undertaken to exclude other diagnoses. Increasing awareness of Gaucher disease as a differential of splenomegaly and cytopenias amongst haematologists could shorten the diagnostic time course and reduce the number of unnecessary tissue biopsies.

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Mesenteric and mediastinal lymphadenopathy in Egyptian children with Gaucher disease : A study of 6 patients. Can Enzyme Replacement Therapy(ERT) play a role in improving their condition?

Abdelwahab M*, Seif eldeen H, ElBeshlawy A*, Eid K*, Gouda I***.**

*Department of Pediatric Hematology, Cairo University Pediatric hospital

**Department of Pediatric Radiology, Cairo University Pediatric hospital

***Department of Pathology, National Oncology Institute, Cairo.

Introduction : Gaucher disease(GD) is a lysosomal storage disorder divided phenotypically into non-neuronopathic, type 1 and neuronopathic, type 2 and 3. It is characterized by deposition of Gaucher cells in liver, spleen, bone marrow, lymph nodes, brain and lung. Reports of mesenteric and mediastinal lymphadenopathy in the literature are very few and usually sporadic case –reports of symptomatic children of type 3 GD. Since the start of enzyme replacement therapy(ERT), selected tissues as lymph nodes were reported to have diminished/absent responses . However, other studies showed that the response of lymphadenopathy to treatment is not known.

Aim : to report this rare finding in 6 children with Gaucher disease and determine the response of lymphadenopathy to replacement therapy in our patient group

Methods : Diagnosis of GD was confirmed by decreased leukocyte β – glucocerebrosidase activity and molecular study was done with full sequencing of the glucocerebrosidase gene .All patients were started on imiglucerase at an initial dose of 60 U/kg/2 weeks with routine laboratory and sonographic follow up every 6 months .When patients developed gastrointestinal manifestations and /or on accidental discovery of abdominal masses, Computerized Tomography(CT)-abdomen with CT-guided biopsy was done.

Results : Here we report six children with Gaucher disease from 4 families of consanguineous marriage who developed mesenteric and mediastinal lymphadenopathy due to infiltration with Gaucher cells after starting ERT for a mean period of 3 years. Three children had type 1 GD (R359Q/R359Q , 2 unknown genotype)and 3 type 3 GD(L444P/L444P) with age ranging from 7 to 11 years. Four were symptomatic showing some clinical improvement and even radiological in a couple but that was dose dependent. Other system involvement included bony complications, neurological manifestations and hepatic fibrosis in one patient. In conclusion, infiltration of lymph nodes with Gaucher cells is one of the complications of the disease that need to be looked for in the pediatric age group and its relation to disease and therapy duration, dosing of ERT , genotype as well as other system involvement need to be further evaluated for better management of those patients .

Gaucher disease and malignancies: Diagnostic and therapeutic challenges.

Rosenbaum H.

MD Department of Hematology & Bone Marrow Transplantation; Rambam Health Care Campus and Bruce Rappaport Faculty of Medicine, Technion, Institute of Technology, Haifa Israel.

Background : Association between Gaucher disease (GD) and malignancy was suggested in several reports. Lymphoproliferative disorders (LPD) and Multiple myeloma (MM) were the most common coexistent neoplasms with GD. In some patients more than one malignant disorder was diagnosed during the course of GD. Diagnosis of LPD and MM in GD patients is challenging due identical constitutional symptoms and difficulty in identifying myeloma and Lymphoma cells in the bone marrow infiltrated with Gaucher cells(GC), requiring immunohistochemistry, immunophenotyping, molecular and cytogenetic evaluations. Lymphadenopathy caused by GC infiltration may mimic lymphoma. Bone complications in GD and MM may be similar, hindering the differential diagnosis of the two states.

Goals : Describe diagnostic and treatment approaches in patients with concomitant GD and solid tumors, lymphoma and MM.

Methods : Data of 160 GD patients followed at the GD clinic of the Rambam Medical Center were analyzed aiming to evaluate the incidence of malignancies, and delineate diagnostic and management options in patients with concomitant GD and cancer.

Results : Among 160 GD patients, 27(16.8%) were diagnosed with malignancies. 12(7.5%) had solid tumors, 7(4.3%) LPD 6(3.7%)MM and 2(1.25%)myelodysplastic syndromes. One GD patient suffered from Waldenstrom macroglobulinemia and renal cell carcinoma. Most of the patients with hematological malignancies were treated with enzyme replacement therapy (ERT) and chemotherapy protocols aiming to achieve hematological remissions with only mild myelosuppression. In cases of GD patients with solid tumors special therapeutic measures were taken to enable surgery and adjuvant chemotherapy considering thrombocytopenia and leucopenia. In one patient with MM successful autologous peripheral stem cell transplantation was performed. In a patient with metastatic colon carcinoma and persistent thrombocytopenia an embolisation procedure of the splenic artery was performed enabling successful removal of liver metastases.

Conclusions : GD appear to be associated with a high incidence of malignancies. Diagnosis and treatment are challenging, given the presence of Gaucher cells in the bone marrow, interfering with identification of malignant cells in bone marrow and lymph nodes in hematological neoplasms, and resulting in pancytopenia, which makes chemotherapy more complicated. Therapy should include chemotherapy and early ERT to prevent prolonged pancytopenia in GD patients with cancer.

β -Glycosphingolipids as immune modulators for oral immune therapy

Yaron I.

- Oral immune therapy is an approach to treat autoimmune, infectious, malignant and inflammatory diseases. It is an active process that uses the inherent ability of the GI tract's immune system to control unwanted systemic immune responses, by inducing regulatory T cells in an antigen-specific manner.
- Oral administration of β -glycosphingolipids skews the immune profile and exerts an immune modulatory beneficial effect in incongruous models. The effect of β -glycosphingolipids may be associated with promotion of the DC - NKT interaction, and by alteration of lipid rafts and intracellular signaling. β -glycosphingolipids can serve as potent adjuvants for oral immune therapy.

Long-term bone mineral density response to enzyme replacement therapy in a retrospective pediatric cohort of Gaucher patients.

Ciana G, Deroma L, Franzil AM, Dardis A, Bembi B.

Regional Coordinator Centre for Rare Diseases, University Hospital “Santa Maria della Misericordia”, Piazzale Santa Maria della Misericordia 15, 33100 Udine, Italy.

A. M. Franzil

Pediatric Department, Ospedale degli Infermi, Via Caraccio 5, 13900 Biella, Italy.

Abstract

Osteopenia is described as a relevant sign of bone involvement in Gaucher disease (GD) both in pediatric and adult patients. Furthermore, abnormal bone metabolism is considered to play a role in growth and pubertal delay. To analyze the long-term effect of enzyme replacement therapy (ERT) on bone mineral density (BMD), a retrospective observational study was conducted in a cohort of 18 GD pediatric patients (13 males, 5 females; median age 9.2 years). They received biweekly infusions of 20-60 IU/kg of alglucerase/imiglucerase. Clinical, laboratory and imaging parameters were evaluated every 2 years. According to the International Society of Clinical Densitometry guidelines, a Z-score ≤ -2.0 was considered pathological. Nine patients (group P0) began ERT during infancy and nine (group P1) during puberty. At baseline, in three patients (16.6 %; 1P0, 2P1) Z-score was ≤ -2.0 (range -2.47 to -2.25). In patient P0 it normalized after 2 years, while in the 2P1 patients (splenectomised siblings) it persisted abnormal. The remaining 15 patients (83.4 %) always presented a normal value. In group P0, Z-score improved in infancy but showed a significant decrease during puberty, on the contrary it constantly improved in group P1. Furthermore, at baseline group P0 showed a higher median Z-score than group P1: 0.79 (0.38; 1.50) and -1.61 (-2.25; -1.56) respectively. The use of correct BMD standards to interpret bone loss during pediatric age suggests a limited significance of bone loss in these patients. Moreover, the persistence of residual disease activity may affect normal bone growth during puberty in GD populations.

Glucocerebrosidase deficiency in zebrafish leads to primary osteogenic defects.

Zancan I^{1,3}, Filocamo M², Hammond C⁴, Argenton F¹, Moro E³.

¹ Department of Biology, University of Padova, Italy.

² Lab. Diagnosi Pre-Postnatale Malattie Metaboliche - IRCCS G. Gaslini, Genova, Italy.

³ Department of Biomedical Sciences, University of Padova., Italy.

⁴ Department of Biochemistry, Physiology & Pharmacology, University of Bristol, BS8 1TD, UK.

Introduction : Osteopenia and other skeletal complications have a considerable influence on the morbidity of patients affected by Gaucher disease (GD). Despite the development of novel therapeutic approaches, bone response to current enzymatic replacement is slow and bone manifestations may worsen or persist in affected patients. The pathogenetic mechanisms responsible for bone alterations are currently unknown.

Aim : This study was aimed to analyze bone defects occurring in a fish model with a morpholino-induced glucocerebrosidase (GBA) deficiency. Moreover, key molecular pathways affected by GBA loss of function were investigated.

Methods : We used a set of transgenic biosensor fish to identify the involvement of targeted cell signaling pathways as a consequence of GBA deficiency. Confocal live imaging on cartilage and bone specific transgenics and transcriptomic analysis were additionally used to perform a detailed spatiotemporal characterization of key molecular genes affected by GBA loss of function.

Results : Our study suggests that well-defined osteogenic defects occur during early life stages in fish larvae lacking correct glucocerebrosidase functional activity. Moreover, when testing a set of biosensor reporter fish, we found a specific impairment of signaling pathways, which are good candidates in bone formation.

Discussion : An early onset of cell signaling alterations detected in our fish model supports a view that GBA loss of function leads to premature primary bone defects, which significantly compromise bone remodeling in later stages. This study emphasizes the use of an early therapeutic intervention in GD affected children and suggests potential novel key targets for therapy of the skeletal disorders in GD.

Glucocerebrosidase deficiency in mesenchymal stem cells from a cohort of 10 Gaucher Disease type 1 patients leads to abnormal osteogenesis.

Lecourt S¹, Belmatoug N², Heraoui D², Mouly E³, Arnulf B^{3,4}, Larghero J¹.

¹ Assistance Publique-Hôpitaux de Paris, Hôpital Saint Louis, Unité de Thérapie Cellulaire et Centre d'Investigation Clinique en Biothérapies CIC-BT501, Paris, France ; Univ Paris Diderot, Sorbonne Paris Cité, F-75475 Paris, France.

² Assistance Publique-Hôpitaux de Paris, Hôpital Beaujon, Service de Médecine Interne, Centre de Référence pour les Maladies Lysosomales, Clichy, France.

³ EA3963, Univ Paris Diderot, Sorbonne Paris Cité, Institut Universitaire d'Hématologie, 75475, Paris, France.

⁴ Département d'Immuno-hématologie, Hôpital Saint-Louis, 75475, Paris, France.

Introduction: Gaucher disease (GD) is an autosomal recessive disorder characterized by lysosomal glucocerebrosidase (GBA) deficiency leading to hematological and skeletal manifestations. Mechanisms leading to these symptoms are poorly explained. Bone marrow (BM) mesenchymal stem cells (MSCs) should represent a cell population involved in the development of bone and hematological disease in GD as they represent multipotent progenitors that participate in the regulation of bone mass and that support hematopoiesis. In a recent study, Campeau et al reported, in a type 1 GD patient, that MSCs displayed an altered inflammatory secretome.¹ These data have been recently confirmed in a mouse model of GD which displayed skeletal manifestations partly related to an impairment of BM MSCs properties.²

Aim: Pathophysiology of bone and hematological abnormalities observed in GD have not yet been understood, thus, we hypothesized that GBA deficiency may induce modification in the MSCs function. In this study, we aimed to characterize the function of GD patients BM microenvironment.

Methods: In a series of 10 patients with type 1 GD, we prospectively characterized BM-MSCs and investigated their capacity to differentiate into osteoblasts. We also analyzed MSCs potential to support long-term hematopoiesis and characterized hematopoietic stem/progenitors cells (HSPCs) intrinsic function.

Results & discussion: GBA deficiency in MSCs from GD patients leads to an impairment of proliferation and to morphological abnormalities. The capacity of MSCs to differentiate into osteoblasts was decreased; furthermore, MSCs secrete soluble factors that increase osteoclasts bone resorption. *In vitro* and *in vivo* primitive and mature hematopoiesis, were similar between patients and control CD34⁺ cells. However, MSCs derived from GD patients had a lower hematopoietic supportive capacity than those from healthy donors. These data suggests that BM microenvironment may be affected in GD and that MSCs are key components of the manifestations observed in GD.

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Taliglucerase alfa leads to favorable bone marrow responses in patients with type I Gaucher disease.

van Dussen L¹, Zimran A², Akkerman EM⁷, Elsein D², Aerts JM.³, Petakov M⁴, Rosenbaum H⁵, Aviezer D⁶, Brill-Almon E⁶, Chertkoff R⁶, Maas M⁷, Hollak CEM¹.

¹Department of Endocrinology and Metabolism, Academic Medical Center, University of Amsterdam, the Netherlands; ²Shaare Zedek Medical Center, Hebrew University and Hadasha Medical School, Israel; ³Department of Medical Biochemistry, Academic Medical Center, University of Amsterdam, the Netherlands; ⁴Clinical Center of Serbia, Serbia; ⁵Rambam Medical Center, Israel; ⁶Protalix Biotherapeutics, Israel, ⁷Department of Radiology, Academic Medical Center, University of Amsterdam, the Netherlands.

Taliglucerase alfa is a carrot-cell-expressed recombinant beta-glucocerebrosidase formulation developed as a treatment for Gaucher disease (GD). In a pivotal, double-blind, randomized Phase III study, PB-06-001 and its extension study PB-06-003, designed to evaluate safety and efficacy of Taliglucerase alfa, two dose levels, 30 and 60 units/kg biweekly, were compared in treatment naïve patients (1). As bone disease is one of the most debilitating features of GD, quantification of bone marrow involvement is of importance for monitoring the response to treatment. For this purpose, bone marrow fat fraction (Ff) measured by Quantitative Chemical Shift Imaging (QCSI) was included as an exploratory parameter in a subpopulation of this cohort.

Aim: To assess bone marrow response by QCSI in GD patients treated with taliglucerase alfa up to 36 months in relation to overall response and antibody status.

Methods: Eight GD patients with intact spleens were treated with 60U/kg (n=4) or 30 U/kg (n=4) biweekly. QCSI results were compared to results in 15 untreated GD patients with a follow-up of at least 1 year extracted from the Dutch Gaucher database.

Results: Five taliglucerase treated patients had a decreased Ff (<23%) at baseline (median (n=8): 19%, range 11-35%). Ff significantly increased compared to baseline (p = 0.012) and compared to untreated patients (p = 0.007) already after 1 year of follow-up with further improvement up to 36 months (median absolute increase at max. follow-up 13.5% (range 5-29%). In four patients with the lowest Ff the higher dose resulted in increases above 23% within one year. All patients had sustained improvements in all other parameters. There was no influence of antibodies on response parameters.

Conclusion: Treatment with taliglucerase alfa results in significant increases in lumbar spine fat fractions, which indicates clearance of Gaucher cells from the bone marrow.

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Pathophysiology of skeletal manifestations in type 1 Gaucher disease.

Lafforgue P.

Hôpital Sainte-Marguerite, CHU de Marseille, France.

Skeletal manifestations are both a major clinical component and the main cause of disability in type 1 Gaucher's disease. Bone deformity (Erlenmeyer flask deformity of the femur) is benign and its pathophysiology remains hypothetical. Ischemic bone necrosis frequently occurs, as bone infarcts or as epiphyseal osteonecrosis. Bone infarcts may occur anywhere in the skeleton, and may be asymptomatic or cause acute painful bone crises. Epiphyseal osteonecroses affect mainly the femoral heads and the proximal humeri. They often result in joint destruction and subsequent prosthetic surgery. They can occur at anytime of the disease course. Risk factors of osteonecrosis are not well established, but this complication is more frequent in patients with other skeletal involvement and in splenectomized patients. Enzyme replacement therapy (ERT) decreases the incidence of bone crises and osteonecroses but does not totally prevent them. A less frequent bone complication is fragility fractures of the long bones or of vertebral bodies. They are facilitated by focal osteolytic lesions (especially at the limbs), and by low bone mineral density (especially at the spine). Indeed, Gaucher disease is associated with a mineral bone density lower than that of the general population, and as people live longer, they can also have age- or menopause-related osteoporosis. ERT is associated with partial improvement of BMD. The place of bisphosphonates in the treatment of Gaucher's disease bone involvement is not well established. Finally, skeletal involvement in Gaucher disease also include growth retardation and osteomyelitis. Although the intimate mechanisms of all these manifestations remain largely unknown, it is clear that bone marrow replacement with Gaucher's cells is the initial common cause.

Abnormal hemorheological and adhesion properties of red blood cells in Gaucher disease.

Franco M, Collec E, Connes P, Billette de Villemeur T, Belmatoug N⁴ Hermine O⁵, Colin Y¹, Le Van Kim C¹, Mignot C^{3,4}

Inserm U665, 75015 Paris, France, Hôpital Trousseau, 75012 Paris, France, Hôpital Beaujon, 92110 Clichy, France, Hôpital Necker, 75015 Paris, France.

Gaucher disease (GD) is a lysosomal storage disorder caused by glucocerebrosidase deficiency, impairing glucosylceramide (GCer) catabolism and resulting in the abnormal accumulation of GCer in macrophages. GD symptoms include moderate anemia, vascular occlusion, avascular necrosis (AVN), and spleen and bones infarcts. Clinical use of the recombinant enzyme as treatment showed improvement in GD manifestations. The hypothesis is that the underlying mechanisms of GD complications might be related to abnormal red blood cell (RBCs) properties. Non-treated Gaucher RBCs may have impaired morphology, vascular adhesion and abnormal rheology that could trigger vaso-occlusion events.

The present study compared the morphology as well as the adhesion and rheologic properties of RBCs from non-treated GD patients (NTGD RBCs) and healthy subjects (CTR RBCs). RBCs flow dynamic adhesion experiments were conducted on microvascular endothelial cells (HMEC-1) and purified laminin. Lu/BCAM (Lutheran/Basal Cell Adhesion Molecule, i.e the only erythroid receptor for laminin) expression and phosphorylation state were characterized by flow cytometry and biochemical studies respectively. Ektacytometry (LORCA) experiments were performed in order to characterize deformability and aggregation properties between the two groups.

We demonstrated that NTGD RBCs as compared to control RBCs (CTR) had enhanced adhesion to endothelial cells and laminin (LN), a major component of the extracellular matrix. This increased adhesion was mediated by the Lu/BCAM glycoprotein. We showed that Lu/BCAM expression, as well as its phosphorylation level, were increased in NTGD compared to CTR RBCs. Furthermore, flow adhesion assays on LN combined with Lu/BCAM staining demonstrated the presence of GD RBCs with a “*tether*” shape suggesting some abnormal membrane properties. Consistent with this hypothesis, ektacytometry (LORCA) experiments revealed a reduced RBC deformability at low shear stresses in NTGD compared to CTR RBCs indicating membrane flexibility defect. RBC aggregation properties were different between the two groups. Preliminary results suggest that treated GD patients (TGD) had less RBCs alterations than NTGD patients.

In conclusion, our study revealed several abnormal properties of RBCs in GD patients such as abnormal RBCs morphology, increased adhesion to endothelium and to laminin and altered hemorheology. These RBCs abnormalities might be responsible for vaso-occlusion-like process and AVN in GD.

Histological findings of femoral heads from patients with Gaucher disease treated with enzyme replacement.

Lebel E, Elstein D, Zimran A, Reinus C, Peleg A, Amir G.

Departments of Orthopedic Surgery, Pathology and the Gaucher Clinic, at Shaare Zedek Medical Center; affiliated with the Hebrew University School of Medicine, Israel.

Introduction: Bone manifestations induce morbidity in Gaucher disease. Management includes orthopedic interventions and disease-specific enzyme replacement therapy (ERT). Recently, it was suggested that femoral joint replacements should be postponed until ERT induces reduction in Gaucher cell (GC) infiltration.

Aim: Our purpose was to assess correlations of patient demographics (including ERT) with bone histology to facilitate decisions of whether/when to perform hip replacements.

Methods: We examined histology of surgically-removed femoral heads and categorized findings by presence/extent osteonecrosis, GC infiltration, and bone regeneration qualifiers using a new scoring system.

Results: 22 patients with 26 bone specimens were evaluated. 17 patients (77%) were splenectomized; 16 (73%) were receiving ERT; 12 (55%) had milder Ashkenazi genotype (N370S/N370S), the rest at increased risk for skeletal disease (N370S /other). Osteonecrotic bone was seen in 19/26 (73%); osteoarthritis in all cartilage specimens. GC infiltration was not correlated with demographics or disease severity. A trend was noted between reduced GC infiltration and ERT ($\rho = 0.407$). Bone regeneration qualifiers were not correlated with ERT or other demographics.

Discussion & Conclusions: Histological findings of GC infiltration and bone regeneration qualifiers neither correlated with demographics nor were clearly affected by ERT. Most specimens showed good regenerative responses to osteonecrosis despite heavy GC infiltration.

Bone parameters in adults with type 1 gaucher disease treated with Velaglucerase alfa in trial TKT025 and the extension study : focus on the bone marrow burden scores over 7 years.

Elstein D^a, Haims AH^b, Zahrieh D^c, M Cohn G^c, Zimran A^a.

^aShaare Zedek Medical Center and Hebrew University-Hadassah Medical School, Jerusalem, Israel; ^bYale University School of Medicine, New Haven, CT, USA; ^cShire Human Genetic Therapies, Inc., Lexington, MA, USA

Introduction : The semi-quantitative magnetic resonance imaging (MRI) bone marrow burden (BMB) score is used to evaluate bone marrow involvement and response to enzyme replacement therapy in Gaucher disease (GD). The lumbar spine (LS) and the femurs are imaged and scored; the LS score and the femoral score each range from 0 to 8 (normal [0–1] to severely abnormal [8]).

Aim : To evaluate changes in BMB score in patients with type 1 GD (GD1) treated with velaglucerase alfa in the trials TKT025 and TKT025EXT.

Methods : Non-splenectomised adults with untreated GD1 received 60 U/kg of velaglucerase alfa every other week (EOW) in TKT025 (9 months). Dose reduction was implemented in the extension study TKT025EXT. Skeletal assessments included T1- and T2-weighted MRI and—to measure bone mineral density (BMD)—dual-energy x-ray absorptiometry.

Results : 12 patients received velaglucerase alfa in TKT025 (median age: 39.3 [range: 18.8–69.8] years; 7 female), of whom 10 enrolled in TKT025EXT and 8 completed a total of ≥ 81 months of velaglucerase alfa therapy. Stepwise dose reduction to 30 U/kg EOW began after 15–18 months relative to Baseline (TKT025 entry). At Baseline, 10 of 12 patients were osteopenic or osteoporotic at both the LS and femoral neck (FN), according to WHO criteria regarding BMD T-scores, and the median BMB score for the LS was 6 (range: 3–8). The mean change from Baseline in LS BMB score was statistically significant ($P < 0.05$) at 9 months (-1.8, 95% CI: -2.9, -0.7; $n = 11$); at 57 months (5 years), the mean change from Baseline was -4.3 (95% CI: -5.7, -2.8; $n = 8$), a decrease ≥ 2 points was seen in 8 of 8 patients, and in 7 patients, the score was 1. The 5-year LS scores were the same at 81 months (7 years). MRI of the femurs did not capture the whole femur in many cases; partial views were inadequate for scoring. Each of 3 patients with images evaluable at Baseline and in TKT025EXT showed a 2-point decrease in the femoral score—from Baseline scores 5, 5 and 3—by 5 years, which was maintained over the subsequent 2 years.

Discussion : In this cohort with significant Baseline skeletal pathology, a statistically significant change in the LS BMB score preceded the previously reported improvement in the BMD Z-score for either the LS (24 months) or FN (33 months). By 5 years, the LS BMB score of each patient with available data except 1 had normalised while all showed a clinically meaningful improvement (defined as ≥ 2 points) from Baseline, which remained at 7 years.

Whole body MRI technique in early treated non-neuronopathic patients with enzyme replacement therapy at least eight years.

Moos L, Reinke J, Heidrich A, Brixius-Huth M, Mengel E, Staatz G.

MC Johannes-Gutenberg-University Mainz, Department of Pediatric Radiology and the Villa Metabolica in the Center of Pediatric and Adolescence Medicine.

Introduction: Within the last 2 decades the early recognition of GD has lead to treatment initiation in many GD type 1 patients before the age of 10 years. Hence, the feasibility of a permanent monitoring of the patient is secured over years. Especially for illustration of bone marrow response to ERT over time the whole body MRI technique may improve our monitoring protocol.

Aim: To show the outcome of early treated patients with treatment initiation before the age of 10 years after 8 – 20 years of ERT, when they become young adults.

Subjects & methods : In this study of the Mainz cohort we include 14 patients in the age of 17 – 26 years with respect to the inclusion criteria treatment initiation before the age of 10 years. All adult patients were confirmed of a non-neuronopathic GD diagnosis and are treated since a minimum of eight years with the Mainz treatment protocol. A whole body MRI was carried out by thirteen patients during work-up routines. A standard MRI-protocol was executed to each of them. We used the bone marrow burden (BMB) score described by Maas et al. (2001). Also the Düsseldorfer Gaucher Score (DGS) applied by Poll et al. (2003). Last analyzing scoring system executed is the Vertebra-disc-ratio by Vlieger et al. (2002). Further the Severity Score Index Type 1 (GD-DS3) is executed according the publication from Weinreb et al. 2010.

Results : All whole body MRI's were well tolerated from the patients. The median BMB is 7 (range 3-11), Q₂₅ is 6/Q₇₅ is 8, 26. Median DGS is 4 (range 3-5); Q₂₅ is 4/Q₇₅ is 4, 5. Median VDR is 1, 33 (range 1, 19-2.01); Q₂₅ is 1, 05/Q₇₅ is 1, 59. Median GD-DS3 is 1, 6 (range 0-2.26); Q₂₅ is 1, 6/Q₇₅ is 1, 6.

Discussion : The study shows that bone marrow involvement and typical manifestations of GD summarized in the GD-DS3 score are reduced to a minimum, when ERT started immediately after a confirmed diagnosis of Gaucher type 1. All scores are located in the lower level, excluding the VDR- Score, which is located in the range of an untreated Gaucher patient. This may reflect postponed fat conversion of the juvenile bone marrow. The issue has to been followed in further studies.

The Nurse Experience.

Morris L.

Addenbrooke's Hospital, Cambridge UK.

Although Gaucher disease is usually regarded as the most common lysosomal storage disorder, it remains for the general population an extremely rare disease. Treatments for this ultra orphan disease, whilst highly effective, remain costly and have sparked significant commercial interest as well as the imposition of stringent management criteria by purchasing authorities. This creates a unique role for the nurse, enabling Gaucher sufferers to navigate their way through the complex healthcare system from diagnosis to expert patients.

Nurses can play an integral role in clinical management, clinical trials, education and psychosocial support, whilst enabling patients to live a full and active life¹. In particular nurses can facilitate safe and effective⁴ home treatment which many patients find to be more convenient and less stressful than receiving treatment in hospital². Home treatment however is not widely available throughout Europe despite its advantages of patient satisfaction and cost effectiveness.

Nurses are patient advocates, instrumental in promoting best practice, who regularly look for ways to improve patient care, through working in collaboration with other members of the multidisciplinary team, locally and internationally.

¹Knight J. A special relationship. *Nursing Standard* 2011 June 22:vol 25 no 42 p 16-17

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The patient experience

Timmerman W.

Coping is the term used to describe the behaviours that individuals adopt to deal with issues associated with their disease.

Although modified behaviour patterns do not always permanently resolve these, they do provide a way for individuals to restore a sense of order to their life.

Patients have different ways of coping with illness.

I will tell about how it started in 1992 and how the patient organisation was involved in home treatment.

I will present my own experience, how I deal with it, how the situation has been changed since 1992, how it is today and the coming adjustment.

The unique role of the clinical trial nurse.

Chenzion Y, Rosenbaum H.

Department of Hematology and Bone Marrow Transplantation,
Rambam Health Care Campus, Haifa, Israel.

The clinical trials nurse (CTN) is a new emerging nursing specialty. Modern healthcare systems around the world offer a wide variety of opportunities to nurses involved in clinical research.

The goal of this analysis was to assess the role of the nurse in the clinical study setting. In addition to providing and coordinating medical care, clinical trial nurses play the central role in ensuring safety and compliance of study participants. Ethical considerations and patients' wellbeing are crucial for the research process. To address the inherent challenges of the new specialty, the CTN should possess multiple skills and professional knowledge of a wide range of nursing concepts, research principals and protocols (GCP).

CTNs in Gaucher clinics have added value to the research team due to their ability to contribute basic nursing principles and skills, e.g., clinical and critical thinking, interpersonal skills, bedside experience, patient advocacy, scientific knowledge, and understanding of individual behavior. They are responsible for enlisting, receiving informed consent, giving treatments (intravenous), ongoing maintenance, integrity of protocol implementation, accuracy of data collection, adherence to the protocol, patient retention in the trial, data recording and follow up. Evidence is beginning to emerge demonstrating how CTN involvement can improve clinical trials and patient outcomes. Anecdotal data presented in the study analysis performed by Pitler et al (2009) suggest that CTN participation in research is associated with improved quality of communication, as well as increased recruitment of participants and improved adherence to protocols. Nurses play a central role in patient care as the largest professional group working in the health system. Gaucher nurses are in an ideal position to promote their patients' awareness of the role played by clinical trials in the advancement of their health and the subsequent improvement in their quality of life.

For me, after over 28 years of clinical nursing, this new evolving professional field is extremely exciting. Bringing scientific knowledge to the bedside, being able to see how the life of Gaucher patients has been totally transformed through new treatments is immensely gratifying.

**Design of a therapeutic patient education for Gaucher's disease patients:
The French experience.**

**Serratrice C, Mamine S, Stirnemann J, Iazza M, Paillet C, Toussaint E, Genevaz G,
Lapointe AS, Lassire JL, Maguars Y, Belmatoug N on behalf of the CETG.**

CETG : Comité d'Evaluation du Traitement de la maladie de Gaucher, Beaujon Hospital,
100 Bd Général Leclerc, 92110 Clichy

VML : Vaincre les Maladies Lysosomales, 2 Ter avenue de France, 91300 Massy

Therapeutic patient education (TPE) is an essential part of chronic disease care, enabling to achieve autonomy in health-related decisions, a better quality of life and better health outcomes. TPE is one of the missions of French CETG. In order to elaborate an educational program adapted to patients, a working group was made up including physicians, nurses, psychologists and the patient's association VML.

The first step was an evaluation of the patient's needs based on patients and professionals' interviews. This investigation brought to light a plurality of representations and real-life experience of the disease as well as numerous repercussions on the working life, the social life, the balance of the couple and the family. Furthermore the burden of the fatigue and the pain were underlined by the patients.

Several preferential themes have been identified to design the educational program which contains:

- (1) A personalized consultation in order to identify the patient needs (educational diagnosis) and to organize his educational training
- (2) Three educational group sessions of approximately 2 hours. The choice of sessions for a given patient will be negotiated by the physician and the patient together. They concern the following themes:
 - a) Better understanding of Gaucher disease and its consequences (definition, symptoms, natural evolution, follow-up and complications)
 - b) Living with the disease in everyday life (real-life experience, fatigue and pain management, identification of available resources)
 - c) Better appropriation of his (her) treatment (criteria of choice of the specific treatment, additional treatments, oral treatment and drip administration)
- (3) A follow-up consultation, support and evaluation of patient's progress

The program is based on interactive educational methods meeting quality criteria of adult's training and it is accompanied by varied educational tools.

In the future this program will be submitted to the regional health agency ; the respective teams concerned will receive training in therapeutic education; the implementation will be the object of an evaluation study (feasibility, satisfaction, profits in skills and patients quality of life)

The group is facilitated by éduSanté with the support from Genzyme.

A Phase 2 multicenter, open label, switch over trial to evaluate the safety and efficacy of Abcertin[®] (Imiglucerase) in patients with type 1 Gaucher disease previously treated with Imiglucerase.

Wook Yoo Han, Hee Lee Beom, Jin Sung Lee Ko Jung Min, Bae Sohn Young.

Medical Genetics Center, Department of Pediatrics, Asan Medical Center Children's Hospital.

Department of Clinical Genetics, Severance Children's Hospital.

Department of Pediatrics, Seoul National University Children's Hospital.

Genetics Clinic, Department of Medical Genetics, Ajou University Hospital.

Introduction: The Gaucher disease type 1 is the prototype of LSD where enzyme replacement therapy proved to be effective. This switch over clinical trial was undertaken to evaluate the safety of Abcertin[®] for patients receiving Imiglucerase therapy for type 1 Gaucher disease.

Aim: The primary objective of this study aimed at evaluating the safety. The analyses of efficacy on changes in hemoglobin, platelet, liver, spleen, biomarkers, skeletal status and BMD were the secondary endpoints.

Methods: Five Korean type I Gaucher patients previously treated with Imiglucerase have been enrolled. The previous Imiglucerase dose ranged from 15 to 60 U/kg with an every other week dosing regimen. Each patient was required to be on the same Imiglucerase dose for at least six months prior to enrollment. A patient's Abcertin[®] dose should be the same dose as the previous Imiglucerase dose administered for immediate past six months.

Results: No clinically significant adverse event was observed. In addition, no changes in the hemoglobin concentration and platelet counts, liver and spleen volumes, and skeletal status and bone mineral density were found. No patient required a dose adjustment. All the patients did not generate antibodies to Abcertin[®]. As the efficacy and safety of Abcertin[®] were demonstrated as similar to those of Imiglucerase, study drug Abcertin[®] could be used as an alternative therapeutic agent in patients who are treated with Imiglucerase.

Discussion: Even though small numbers of patients were recruited, this was the first trial to investigate the safety and efficacy of Abcertin[®]. Another clinical trial is under way to verify the efficacy and safety in naïve type I Gaucher patients and the data will be available soon.

ZAGAL Study : Long-term management and follow-up of use of Miglustat in type 1 Gaucher disease in Spain.

Giraldo P^{1,2,3,4}, Alfonso P^{2,3,4}, Irun P^{3,4,5}, Atutxa K⁶, Fernández-Galán MA⁶, Barez A⁶, Franco R⁶, Roig I⁶, Giner V⁶, Villalon L⁶, Martinez E⁶, Luño E⁶, Loyola I⁶, Salamero O⁶, de la Serna J⁶, Pocovi M^{3,4,5}.

¹Hematology Department. Miguel Servet University Hospital. Zaragoza. Spain. ²Traslational Research Unit. Miguel Servet University Hospital. Zaragoza. Spain. ³Aragon Health Sciences Institute (I+CS). Zaragoza. Spain. ⁴Biomedical Network Research Center on Rare Biology and Diseases (CIBERER), ISCIII. Zaragoza. Spain ⁵Department of Biochemistry and Cellular and Molecular Biology. University of Zaragoza. Spain, ⁶Working Spanish Group on Gaucher Disease.

The ZAGAL study was started in May 2004 for monitoring the real-life use of miglustat ('ZavescaTM') in Spanish adult patients with mild-to moderate disease unable or unwilling to receive enzyme replacement therapy (ERT). The study included GD1 patients naïve to therapy as well as patients who have previously been treated with ERT.

We have reported previously the results at 24 and 48 months that showed that most patients achieved and maintained the therapeutic goals. In GD1 naïve patients, miglustat showed similar short term efficacy than naïve patients treated with ERT. Long-term data on changes in organ size, blood counts, disease severity biomarkers, bone marrow infiltration, overall clinical status and safety/tolerability in treatment naïve as well as in patients transitioned from ERT, shows that disease severity biomarkers improved up to 48 months after initiation of miglustat, while other disease parameters remained stable. The tolerability profile of miglustat is well established. Its main unwanted effects are mild to moderate and include abdominal bloating and diarrhoea (related to inhibition of intestinal disaccharidases, particularly sucrase and maltase), as well as a tremor and weight loss.

We present the long-term follow-up of GD1 patients that have been treated with miglustat in Spain. To date a total of 302 GD1 patients have been diagnosed in Spain (Orphanet J Rare Dis. 2012); from 2004 until 2011, 52 of them have been exposed of miglustat (17.2%).

Currently 42 patients are on miglustat therapy (15 patients for more than 7 years). In all of them, the therapeutic goals (normalized blood counts, reduced liver and spleen volume and absence of bone crisis) have been achieved or maintained. Fourteen patients had transitory gastrointestinal disturbances. Ten patients discontinued therapy: 3 died by therapy non-related causes (2 neoplasm and 1 hearth attack), one because she was planning to become pregnant and the remaining 6 because of gastrointestinal discomfort or intolerance.

Conclusion : Long term treatment with Miglustat achieves and/or maintains the therapeutic goals in mild or moderate GD1 patients. Treatments tolerability is good. A high individual variability had been observed related to miglustat gastrointestinal intolerance apparently non related with GBA genotype, gender and age, but possibly associated with food habits.

Eliglustat, an investigational oral therapy for Gaucher disease type 1: Phase 2 results after 4 years of treatment.

Lukina E¹, Watman N², Dragosky M³, Pastores GM⁴, Arreguin EA⁵, Rosenbaum H⁶, Zimran A⁷, Sysoeva E¹, Aguzzi R⁸, Ross LH⁸, Puga AC⁸, Peterschmitt MJ⁸.

¹Hematology Research Center, Moscow, Russia; ²Hospital Ramos Mejia, Buenos Aires, Argentina; ³IMAI-Research, Buenos Aires, Argentina; ⁴New York University, New York, USA; ⁵Instituto Mexicano del Seguro Social Hospital de Especialidades, Col. La Raza, Mexico; ⁶Rambam Medical Center, Haifa, Israel; ⁷Sha'are Zedek Medical Center, Jerusalem, Israel; ⁸Genzyme, Cambridge, MA, USA.

Introduction: In Gaucher disease type 1 (GD1), deficient acid β -glucosidase activity causes glucosylceramide accumulation primarily in tissue macrophages (Gaucher cells) and results in multisystemic manifestations, including thrombocytopenia, anemia, hepatosplenomegaly, and bone disease. Eliglustat, a potent and specific inhibitor of glucosylceramide synthase, is under development as an oral substrate reduction therapy for GD1.

Aim: Report long-term efficacy and safety results.

Methods: This ongoing, open-label, uncontrolled, multicenter Phase 2 clinical trial enrolled 26 adults with GD1 not on treatment for the previous 12 months, who had splenomegaly with thrombocytopenia and/or anemia. Efficacy outcomes were assessed periodically and included changes from baseline in hemoglobin, platelets, spleen and liver volumes, skeletal manifestations, biomarker levels, and achievement of therapeutic goals (Pastores, *Semin Hematol*, 2004).

Results: Nineteen patients completed 4 years of eliglustat treatment; no patient discontinued in the last 2 years. After 4 years, mean hemoglobin level and platelet count increased by 2.3 ± 1.5 g/dL (from 11.3 ± 1.5 g/dL to 13.6 ± 1.2 g/dL) and 95% (from $68,700 \pm 21,200/\text{mm}^3$ to $125,400 \pm 51,100/\text{mm}^3$), respectively; mean spleen and liver volumes (multiples of normal, MN) decreased by 63% (from 17.3 ± 9.5 to 6.1 ± 3.4 MN) and 28% (from 1.7 ± 0.4 MN to 1.2 ± 0.3 MN), respectively. All patients met ≥ 3 of 4 long-term therapeutic goals (spleen, 100% of patients; liver, 94%; hemoglobin, 100%; platelets, 50%). Median chitotriosidase and CCL-18 each decreased by 82%; plasma GL-1 and GM3 normalized. Mean lumbar spine bone mineral density increased by 0.8 T-score (from -1.6 ± 1.1 to -0.88 ± 1.3), with the greatest increases occurring in patients with osteoporosis at baseline. Femur dark marrow, believed to reflect Gaucher cell infiltration into bone marrow, was reduced or stable in 17/18 patients. There were no bone crises. Eliglustat was well-tolerated. Most adverse events (AEs) were mild and unrelated to treatment. Ten drug-related AEs, all mild, occurred in 8 patients. No new serious AEs were reported in any patient between 3 and 4 years of treatment.

Discussion: Eliglustat continues to show promising efficacy and safety, with clinically meaningful improvements across disease parameters. Results from two controlled Phase 3 studies in untreated and enzyme replacement therapy maintenance patients will be available in 2013.

Velaglucerase as therapy for Gaucher disease

Elstein D.

Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem Israel

This presentation will review the development of velaglucerase alfa (VPRIV; Shire HGT, Cambridge MA, USA) using a proprietary mannose-targeted, human cell system of recombinant enzyme with the wild type glucocerebrosidase sequence from pre-clinical studies to approval for commercial distribution in 2010.

The results of the clinical trials and extension studies show an excellent safety profile with efficacy data that is early and robust in treatment-naïve and switch-over patients, children and adults, patients with an intact spleen or splenectomized, and in patients with abnormal severe symptomatic involvement at advent of velaglucerase alfa treatment.

Certain themes will be highlighted such as the drug's low immunogenicity; minimal adverse events; efficacy at doses 30-60units/kg body weight/every-other-week; the ability to quickly correct anemia (the primary entry criterion and endpoint in the seminal trials); the significant improvement in platelet counts even in patients heretofore considered 'poor responders'; and the early response of improved bone density and bone marrow burden scores in patients of various ages and with varying degrees of skeletal involvement at baseline. Mention will be made of the patients enrolled in an Early Access Program for 6-12 months and the putative effect among several switch-over patients to develop a new trajectory of improvement after switching to velaglucerase alfa.

Incidence of Parkinson disease in obligate carrier relatives of patients with Gaucher disease: a single-center report.

Dinur T, Elstein D, Zimran A.

Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem Israel.

Introduction: Individuals who have one or two mutations in the β -glucocerebrosidase gene have an increased risk for Parkinson disease (PD).

Aim: To present the incidence of PD among obligate carrier relatives of patients with Gaucher disease (GD) based on personal interviews of patients from a large Israeli GD clinic.

Methods: All patients arriving at the referral Gaucher Clinic in Jerusalem during the period of mid-October 2011-mid May 2012 (6 months) were interviewed with regard to PD in any relative.

Results: 140 patients (with 280 obligate carrier relatives) were interviewed, also representing 30 additional family members with Gaucher disease who are followed at the Gaucher Clinic. There were 5% obligate carriers (14/280) with PD compared to incidence of 2.2% (11/510) of GD+PD patients in the clinic. Another 5% (14/280) had other relatives with PD. One obligate carrier relative of ten patients (20 obligate carriers) with GD+PD (5%) also had PD. Mean age of onset PD among obligate carrier relatives was 66.4 (range: 45-81) years which was later than age onset PD in GD+PD patients, 57.5 (range: 43-75) years. There was no preponderance of males (50%) among obligate carrier relatives with PD as versus 82% (9/11) males among GD+PD patients. At least 9/15 (60%) obligate carrier relatives with PD had the N370S mutation and one had the C245T mutation; 6/11 (55%) of GD+PD were N370S homozygotes.

Discussion: This is the first report of the incidence of PD among obligate carriers of Gaucher disease that implicates a higher incidence of PD than among GD patients, with later age of onset of PD, high prevalence of the N370S mutation, but equal incidence in males and females.

Gaucher disease : from ERAD of mutant glucocerebrosidase variants to Parkinson disease.

Bendikov-Bar I, Horowitz M.

Department of Cell Research and Immunology, Tel Aviv University, Ramat Aviv, Israel.

Mutations in the glucocerebrosidase (GCase)-encoding gene (GBA) lead to accumulation of glucosyl-ceramides, manifested as Gaucher disease (GD). Mutant GCase variants present variable degrees of ER retention and undergo ER associated degradation (ERAD) in the proteasome. The ERAD process requires specific E3 ligases, which mediate ubiquitination of the misfolded enzyme before its elimination in the proteasome.

Gaucher mutations have been recently identified as a major cause for Parkinson disease. One of the genes associated with Parkinson disease is PARK2, encoding the E3 ligase parkin. We tested the possibility that the association between Gaucher disease and Parkinson disease reflects the fact that parkin is an E3 ligase of mutant GCase variants. Our results showed that endogenous mutant GCase undergoes polyubiquitination and proteasomal degradation. Overexpression of parkin in skin fibroblasts that originated from GD patients decreased the amount of mutant, but not normal, GCase, implying that parkin participates in elimination of mutant GCase.

Occupation of parkin with mutant GCase decreases degradation of the natural substrates of parkin, accumulation of which may contribute to cell death. We tested the effect of mutant GCase on a pathway regulated by the parkin substrate PARIS. PARIS is a transcription repressor that undergoes parkin mediated degradation in the proteasome. When it is not degraded by parkin, it accumulates in the nucleus and downregulates expression of genes essential for cell viability. We were able to show decrease in mRNA level of genes downregulated by PARIS, which are important to mitochondrial biogenesis in skin fibroblasts originated from Gaucher patients and in SHSY5Y cells overexpressing mutant GCase. Another parkin substrate is the pro-apoptotic protein ARTS. We could show that increased amounts of ARTS lead to accumulation of mutant, but not normal GCase, indicating that there is a competition between ARTS and mutant GCase.

The E3 ligase Itch regulates degradation of mutant glucocerebrosidase.

Maor G, Horowitz M.

Dept of Cell Research and Immunology, Tel Aviv University, Ramat Aviv, Israel.

Inability to properly degrade unfolded or misfolded proteins in the ER leads to ER stress and unfolded protein response. This is particularly important in cases of diseases in which the mutant proteins undergo ER associated degradation (ERAD), like Gaucher disease. Gaucher disease (GD) is a genetic, autosomal recessive disease that results from mutations in the gene encoding the lysosomal enzyme acid- β -glucocerebrosidase (GCase). Gaucher disease has been subdivided to three major forms, with type 2 being the most severe one. Neonate type 2 GD patients may develop Ichthyosis, which presents hyperproliferation of the skin.

Mutant GCase variants undergo ERAD, the degree of which is a major determinant of disease severity. Most ERAD substrates undergo ubiquitination and proteasomal degradation. Since GCase is not a natural substrate for ubiquitination and proteasomal degradation, one would expect that mutant GCase variants will interact with a wide variety of E3 ligases, in different cells.

We tested the possibility that Itch, a known E3 ligase, with a pivotal role in proliferation and differentiation of the skin, recognizes mutant GCase variants and mediates their ubiquitination and degradation. Our results showed that Itch mediates Lysine 48 polyubiquitination and degradation of mutant GCase variants. We have also shown that transcription level of Itch is elevated in skin fibroblasts obtained from type 2 GD patients, implying a growing need for Itch in the tested cells due to the presence of mutant GCase. Itch mediates degradation of Δ Np63, a transcription factor that is responsible for maintaining the proliferative potential of the basal layer of the epidermis. Our results indicated that elevation in the level of mutant GCase correlates with elevation in Δ Np63 levels in the cells, suggesting a competition between Δ Np63, a known substrate of Itch, and mutant GCase.

We hypothesize that the presence of mutant GCase in proliferating cells of the skin and its recognition and degradation by the E3 ligase Itch leads to accumulation of its other substrate Δ Np63. This accumulation leads to change in the homeostasis between differentiation and proliferation and the appearance of Ichthyotic skin is severe, neonate type 2 GD patients.

Psychosines implicated in the pathogenesis of Gaucher disease alter actin dynamics and perturb intracellular lysosomal trafficking.

Roos JCP, Cox TM.

Department of Medicine, University of Cambridge.

Introduction : It has been proposed that metabolites such as the psychosines accumulating in the Gaucher cell are responsible for its unusual phenotype and pathological effects. Previous studies have shown that incubation of dividing cells with lysolipid can result in polyploidy, but the mechanism by which this occurs has remained obscure.

Aim : To identify the effect of β -glucosylsphingosine (psychosine) on the cytoskeleton and membrane trafficking.

Methods : We used pyrene-actin polymerization assays, live-cell confocal imaging of transfected cells and electron microscopy to investigate the effect of lysosphingolipids on the actin and membrane turnover of cultured cells. The effect of lysolipids on actin was interrogated by comparison with jasplakinolide – an actin stabilizing toxin.

Results : We found that diverse cells can be affected by psychosine but that the concentration required was highly variable. Treatment reduces filopodia formation and induces large and small vesicles to form inside the cell which mimic the appearance of Gaucher cells by EM.

15 μ M psychosine causes first LAMP-containing vesicles and then actin to congregate in HeLa cells. LAMP2 expressing membrane storage is associated with translocation of clathrin and AP2 to the centre of the cell whilst the caveolin-mediated endocytic system remains unaffected. Psychosine treated cells recover coated pit formation more slowly following butanol treatment, all suggesting a specific effect on trafficking.

We confirmed an unaltered rate of phagocytosis following treatment with psychosine: the vesicular area engages in rapid endocytosis and is not open to the external environment as shown by ruthenium red staining. Psychosine does not affect actin polymerisation at low concentrations but forms novel macro-bundles at physio-pathological concentrations. We show that stabilisation of actin with jasplakinolide is sufficient to inhibit cytokinesis but not to form the characteristic vesicles associated with Gaucher disease and psychosine treatment.

Discussion : We have captured live-cell images showing that psychosines at pathophysiological levels have profound effects on the composition and distribution of subcellular compartments and can directly affect actin polymerization at high concentrations. This provides potential mechanistic insight into the pathophysiology of Gaucher disease.

Citations : Roos JC & Cox TM. Giant osteoclast formation and long-term oral bisphosphonate therapy. *N Engl J Med.* 2009;360(16):1676-7.

Competition law and Gaucher and other orphan drug pricing.

Hyry HI, Roos JCP, Cox TM.

University of Cambridge.

Introduction : Access to orphan products remains an acute problem in the EU and beyond. While the EU Orphan Regulation has been a success in bringing to the market effective treatments, the Regulation's 10-year marketing exclusivity enables manufacturers to charge what some consider "exorbitant" prices for orphan products. This affects patients and their national health systems who struggle to finance treatments which may soon consume 6-8% of EU healthcare budgets. The current pricing model thus hinders access to treatment and contravenes the aim of the Regulation: namely to create parity of care between common and rare diseases.

Aim : We have, for the first time in the field, analysed whether the pricing of orphan products may be so high as to contravene EU competition law, aimed at protecting consumers. The possibility of such a finding may encourage stakeholders to agree on strategies for improved access.

Methods : We have conducted a legal analysis of EU competition law and an examination of current price setting practice.

Results : We have proposed (BMJ.2010;341:c6471) a novel and equitable solution to the pricing problem through competition law, which, through Article 102 of the Treaty on the Functioning of the EU, protects consumers from abuse of a dominant position by a company. Abuse includes unfair pricing and other unfair trading conditions. While dominance itself is not an offence, the EU places special legal responsibilities on companies in such a position. Further, as a result of their exclusive market share, orphan companies may be classified as super-dominant – in which case their legal responsibility may be even greater. An EU-commissioned study found that orphan drug price-setting is "highly arbitrary": our provisional analysis supports this conclusion. While the finding of abuse is a complex question of fact, we contend that there is a credible general argument for violation of EU law by the current pricing practices.

Discussion : Infringement of competition law has serious consequences, including fines and severance of company operations. While the prospect of an investigation could rapidly drive manufacturers to lower prices, in our view it is critical that a sufficient incentive for developing life-saving drugs be preserved. We propose that constructive negotiation between all stakeholders is needed to develop a sustainable pricing model for orphan medicinal products.

Citations : BMJ.2010;341:c6471. See article for further citations.

Investigation of a novel approach to treat patients with Gaucher disease.

Brady R.

Recent experimentation has provided fresh insight in regard to the pathogenesis of hereditary enzyme deficiency disorders. For many years it was assumed that alterations of the amino acid sequence of glucocerebrosidase such as N370S/N370S and L44P/L444P mutations caused a reduction of the catalytic activity of the enzyme. My colleagues and I recently observed that the decreases of glucocerebrosidase activity were the result of quantitative reductions of the amounts of these enzymes. The mutations code for mis-folded unstable proteins that are rapidly degraded by the protein quality control system. Significant increases of the catalytic activity of glucocerebrosidase with these mutations were obtained in the presence of small molecule inhibitors of histone deacetylase. The effect of such materials on the characteristic manifestations of Gaucher disease and other hereditary metabolic disorders is under investigation.

Activation of the unfolded protein response in Gaucher disease.

Horowitz M, Maor G.

Department of Cell Research and Immunology, Tel Aviv University, Ramat Aviv, Israel.

Gaucher disease, an autosomal recessive disease, results from mutations in the gene encoding the lysosomal enzyme acid β glucocerebrosidase (GCase). Mutant GCase variants undergo ERAD, the degree of which is a major determinant of disease severity. During this process mutant molecules are recognized as misfolded in the ER, and following abortive attempts to refold them, they are retrotranslocated to the cytoplasm, where they undergo polyubiquitination and proteasomal degradation. The presence of mutant molecules in the ER induces ER stress and the unfolded protein response (UPR). This process involves several pathways, leading to activation of genes, whose products ensure attenuation of protein translation in the cells and upregulation in transcription of chaperones and components that take part in ERAD and apoptosis. Increasing evidence suggest that protein misfolding in the ER lumen and alteration in UPR signaling play important roles in the etiology of numerous disease states.

A previous publication noted UPR in GD derived skin fibroblasts (Mu et al., 2008). We have extended this study to show that UPR exists in GD derived skin fibroblasts, manifested by upregulation of the expression of the transcription factor CHOP and the chaperone BiP (Grp78), phosphorylation of eIF2 α and cytoplasmic splicing of Xbp1. Moreover, our results indicated that UPR leads to upregulation of the GBA gene transcription, implying a direct regulation of the UPR related target gene products on transcription directed from the GBA promoter.

To summarize, UPR is activated in GD derived fibroblasts in an attempt to recover from the ER stress, which results from ERAD of mutant GCase.

References

Mu, T.W., Ong, D.S., Wang, Y.J., Balch, W.E., Yates, J.R., 3rd, Segatori, L., and Kelly, J.W. (2008). Chemical and biological approaches synergize to ameliorate protein-folding diseases. *Cell* 134, 769-781.

Exploring bicyclic derivatives of L-idonojirimycin as pharmacological chaperones for the treatment of Gaucher disease.

Alfonso P^{1,2,3}, Andreu V^{2,3}, Navascues J³, Pino-Angeles A^{1,4}, Moya-García AA^{1,4}, García-Moreno MI⁵, Sánchez-Jiménez F^{1,4}, Pocoví M^{1,3,6}, Ortiz Mellet C⁵, García Fernández JM⁷, and Giraldo P^{1,2,3,8}.

¹Biomedical Network Research Center on Rare Diseases (CIBERER), ISCIII. Valencia. Spain. ²Traslational Research Unit. Miguel Servet University Hospital. Zaragoza. Spain. ³Aragon Health Sciences Institute (I+CS). Zaragoza. Spain. ⁴Department of Molecular Biology and Biochemistry. University of Málaga. Spain. ⁵Department of Organic Chemistry. Faculty of Chemistry. University of Sevilla. ⁶Department of Biochemistry and Cellular and Molecular Biology. University of Zaragoza. Spain. ⁷Institute for Chemical Research (IIQ), CSIC-University of Sevilla. Spain. ⁸Service of Hematology. Miguel Servet University Hospital. Zaragoza. Spain.

Introduction: Many of the mutations of the lysosomal acid b-glucosidase (glucocerebrosidase, GC) associated with Gaucher disease (GD) translate into enzymes that retain partial catalytic activity *in vitro* but exhibit impaired cellular trafficking as a consequence of aberrant folding. Current investigational therapeutic strategies for GD include the development of ligands of the enzyme capable of promoting those conformational changes that are required for efficient folding, restoring trafficking. Although somewhat counterintuitive, competitive inhibitors of this enzyme, at subinhibitory concentrations, can increase steady-state lysosomal levels of active GC through this rescuing mechanism, acting as "pharmacological chaperones". At the massive lysosomal substrate concentration, the inhibitor would be replaced from the active site and the metabolic activity recovered. However, most of the pharmacological chaperones under study are iminosugars that behave as broad spectrum inhibitors, inhibiting simultaneously several glucosidases, which represents a serious inconvenient for clinical applications. An additional problem is that iminosugars are not active as pharmacological chaperones for glucocerebrosidase mutations located outside the domain containing the active site and are associated with neurological involvement.

Aim: To present the application of molecules with a high binding specificity towards GC, with a high ratio of chaperone *versus* inhibitor activity and capable of producing an increased in the levels of mutant enzymes associated with GD, including mutations located outside the catalytic domain.

Methods: Different bicyclic derivatives of L-idonojirimycin were designed and chemically synthesized from D-glucose after *in silico* structural analysis and identification of the most favorable molecular features to interact with the active site of glucocerebrosidase. Their chaperone potential was evaluated *in vitro* using a cell model of GD carrying the more frequent mutations in GD, namely N370S and L444P (P201230804).

Results: Results showed an increase in GC activity at various chaperone concentrations, ranging from 1.96 to 4.98 folds for the L444P mutant and from 2.01 to 3.06 folds for the N370S mutant.

Discussion: The use of bicyclic L-idonojirimycin-based pharmacological chaperones could be considered as a therapeutic alternative for GD, mainly in patients with mutations located outside the active site of GC and associated with neurologic involvement.

The pharmacological chaperone AT3375 alone and in combination with recombinant human acid β -Glucosidase for Gaucher disease.

Elfrida BR, Khanna R, Pellegrino L, Soska R, Lun Y, Feng J, Frascella M, E. Ranes B, Guillen Da, Garcia A, Flanagan JJ, Lockhart DJ, Clark SW, Valenzano KJ.

Amicus Therapeutics, 1 Cedar Brook Drive, Cranbury, NJ 08512

Gaucher disease (GD) is a lysosomal storage disease caused by a deficiency in acid β -glucosidase (GCase) activity, and subsequent pathological accumulation of the substrate glucosylceramide (GC) in tissue macrophages. We have shown previously that the small molecule pharmacological chaperone (PC) AT2101 (isofagomine tartrate) binds and stabilizes wild-type (WT) and GCase mutants N370S and L444P, resulting in more efficient cellular trafficking and increased cellular GCase levels. In this study, we investigated the effects of a second-generation PC, AT3375, on endogenous and exogenous GCase *in vitro* and *in vivo*. AT3375 increased WT, N370S, and L444P GCase activity in patient-derived cells with 2- to 5-fold greater potency compared to AT2101. In WT mice, oral administration showed higher brain levels with more rapid clearance of AT3375 compared to AT2101, and increased brain GCase levels with 10-fold greater potency compared to AT2101. Similarly, in L444P GCase mice, 4-week administration of AT3375 showed 2- to 3-fold greater increases in brain L444P GCase levels at lower doses compared to AT2101. Lastly, AT3375 stabilized exogenous recombinant human GCase (rhGCase) *in vitro*, minimizing its thermal denaturation and loss of activity at neutral pH and 37 °C. Importantly, co-administration of AT3375 in rats increased the circulating half-life of rhGCase and led to improved uptake in disease-relevant tissues. Collectively, these data indicate that AT3375 may have potential as a monotherapy for GD with CNS involvement, and may also improve the properties of rhGCase upon co-administration, thus warranting further investigations for its potential in the treatment of GD.

Long-term clinical outcomes in type 1 Gaucher disease following 10 Years of Imiglucerase treatment.

vom Dahl S¹, Weinreb NJ², Goldblatt J³, Villalobos J⁴, Charrow J⁵, Cole JA⁶, Kerstenetzky M⁷, Hollak C⁸.

¹Klinik für Innere Medizin, St. Franziskus-Hospital, Cologne, Germany; ²University Research Foundation for Lysosomal Storage Disorders, Coral Springs, FL, USA; ³Genetic Services & Familial Cancer Program of Western Australia, School of Pediatrics and Child Health, University of Western Australia, Perth, Australia; ⁴Universidad Central de Venezuela, Caracas, Venezuela; ⁵Division of Genetics, Birth Defects and Metabolism, Children's Memorial Hospital, Chicago, IL, USA; ⁶Genzyme, a Sanofi company, Cambridge, MA, USA; ⁷Instituto de Medicina Integral Professor Fernando Figueira, Recife, PE, Brazil; ⁸Department of Internal Medicine, Division of Endocrinology and Metabolism, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Introduction: Evaluation of hematological and organ responses and skeletal manifestations is the standard minimum criterion for evaluating efficacy of treatment for Gaucher disease type 1 (GD1) in both clinical trials and in everyday practice. We hypothesized that imiglucerase therapy would demonstrate a sustained benefit, even after 10 years of treatment.

Aim: To study the effect of long-term alglucerase/imiglucerase (Ceredase®/Cerezyme®, Genzyme, a Sanofi company, Cambridge, MA USA) treatment on hematological, visceral and bone manifestations of GD1 after 10 years of treatment.

Methods: The ICGG Gaucher Registry identified GD1 patients treated with alglucerase/imiglucerase who had dose and clinical data at first infusion and after 10 years of follow-up. Data for hemoglobin, platelet count, organ volumes, bone pain and bone crisis were analyzed. Tests of the null hypothesis (no change from first infusion to 10 years) were performed using t-tests for within-patient absolute change in continuous measurements, and chi-square tests for change in distributions using categorical values. An alpha-level of 0.05 designated statistical significance.

Results: As of October 2011, 557 non-splenectomized and 200 splenectomized patients met the inclusion criteria. The majority of GD1 patients had at least one N370S allele. Compared to non-splenectomized patients at first infusion, splenectomized patients had lower percentages of anemia (26.0% vs. 42.8%) and thrombocytopenia (14.2% vs. 76.3%), similar percentages of moderate or severe hepatomegaly (81.2% vs. 80.0%), and higher percentages of bone pain (88.9% vs. 52.4%) and bone crises (38.3% vs. 16.0%). After 10 years, both groups showed significant ($p < 0.05$) improvements in mean hemoglobin levels, platelet counts, liver, and spleen (non-splenectomized) volumes and bone crises. Initial dosing in both groups ranged from <15 U/kg to ≤ 90 U/kg/2 weeks. After 10 years, the majority were receiving 15 to ≤ 45 U/kg/2 weeks.

Discussion: Clinical improvements in hematological, visceral, and bone manifestations of non-splenectomized and splenectomized patients with Gaucher disease type 1 were maintained after 10 years of imiglucerase treatment.

Plant-cell-expressed recombinant glucocerebrosidase - Taliglucerase alfa as therapy for Gaucher disease in patients previously treated with Imiglucerase.

Pastores GM¹, Szer J², Petakov M³, Cox T⁴, Giraldo P⁵, Rosenbaum H⁶, Amato D⁷, Mengel E⁸, Chertkoff R⁹, Brill-Almon E⁹, Zimran A¹⁰.

¹New York University School of Medicine, Neurogenetics Unit, New York, NY, USA; ²Clinical Haematology and Bone Transplant Service, Royal Melbourne Hospital, 3050 Victoria, Australia; ³Clinical Center of Serbia, Institute of Endocrinology, Diabetes and Metabolic Diseases, Dr Subotica 13, 11000 Belgrade, Serbia; ⁴Department of Medicine, Addenbrooke's Hospital, Cambridge, CB2 2QQ, UK; ⁵Hospital Universitario Miguel Servet, Servicio de Haematologia Planta baja, P Isabel, La Catolica 1-3, 50009, Zaragoza, Spain; ⁶Haematology Ambulatory Services, Rambam Medical Center, 8 Haaliya St., Haifa 31096, Israel; ⁷Mount Sinai Hospital, 600 University Avenue, Room 438, Toronto, Ontario, M5G 1X5, Canada; ⁸Villa Metabolic ZKJM MC Gutenberg-University Mainz, Langenbeckstr. 1 Geb. 907, 1.OG MAINZ, 55131 DEU, Germany; ⁹Protalix Biotherapeutics, Carmiel, Israel; ¹⁰Gaucher Clinic, Shaare Zedek Medical Center, 12 Bayt St, Jerusalem 91031, Israel

Introduction : Taliglucerase alfa is a United States Food and Drug Administration–approved enzyme replacement therapy (ERT) for Gaucher disease (GD). Earlier studies have focused on the safety and efficacy of taliglucerase alfa in treatment-naïve GD patients.¹

Aim : The purpose of this study was to assess the safety and efficacy of taliglucerase alfa in GD patients previously receiving imiglucerase.

Methods : GD patients with clinically stable disease were eligible if they were receiving imiglucerase for ≥ 2 years and were on the same regimen ≥ 6 months. Eligible patients entered a 9-month treatment period and were switched from imiglucerase to taliglucerase alfa at the same dose and received a total of 20 infusions, given every 2 weeks. Each patient's previous historical clinical and laboratory stability measurements served as controls.

Results : Twenty-six adult patients were recruited; 25 completed the 9-month treatment period. Patient ages ranged from 18 to 66 years (mean 47.6 ± 12.9 SD). Nine patients received doses ≥ 30 U/kg, 8 patients received doses >15 U/kg and <30 U/kg, and 8 patients received doses ≤ 15 U/kg. Baseline disease parameters were as follows (mean \pm SD): spleen volume— 6 ± 4.8 multiples of normal (MN; 3 patients were splenectomized); liver volume— 1 ± 0.2 MN; hemoglobin— 13.4 ± 1.7 g/dL; platelet counts— $154,120 \pm 86,550$; chitotriosidase— 7084 ± 9608 nmol/mL*h. Following switchover to taliglucerase alfa, spleen and liver volumes as well as platelet, hemoglobin, and chitotriosidase measurements remained stable or improved. All treatment-related adverse events were mild or moderate in severity and transient in nature; the most commonly occurring were headache (n=2) and infusion-related reaction (n=2).

Discussion : Mean efficacy parameters were maintained and taliglucerase alfa was well tolerated in patients switched from imiglucerase to taliglucerase alfa.

1. Zimran A, et al. *Blood*. 2011;118(22):5767-5773.

A Multicenter, double-blind, randomized safety and efficacy study of two dose levels of Taliglucerase alfa in pediatric subjects with Gaucher disease.

Zimran A¹, Gonzales D², Paz A³, Brill-Almon E³, Chertkoff R³.

¹Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel; ²Instituto Privado de Hematologia E Investigacion Clinica (I.P.H.I.C), Asuncion, Paraguay; ³Protalix Biotherapeutics, Carmiel, Israel.

Introduction: In the treatment of Gaucher disease (GD), early intervention with enzyme replacement therapy (ERT) is crucial in the prevention of irreversible pathology.¹ Taliglucerase alfa is a plant-cell-expressed beta-glucocerebrosidase ERT that is approved in the United States for the treatment of GD in adults.

Aim: To investigate the safety and efficacy of taliglucerase alfa in pediatric GD patients.

Methods: This was a multicenter, double-blind, 12-month study in patients aged 2 to <18 years randomly assigned to taliglucerase alfa 30 or 60 U/kg. The primary efficacy variable was median percent change in hemoglobin concentration from baseline. Secondary variables were the percent change in spleen and liver volumes, platelet count, and chitotriosidase or CCL18. Exploratory endpoints included change in growth and development, bone disease (Dual Energy X-Ray Absorptiometry [DEXA] and occurrence of bone crises) and change in quality of life (QoL). Safety was assessed by clinical laboratory, echocardiography, anti-taliglucerase alfa antibodies, and adverse events (AEs). After completion of the 12-month study, patients were eligible to enter a 2-year extension.

Results: A total of 11 patients (10 type 1 and one type 3) were randomized to taliglucerase alfa 30 or 60 U/kg. Progressive improvement was demonstrated in hemoglobin, spleen volume, liver volume, platelet count, and chitotriosidase activity. At 12 months, composite analysis of both dose groups revealed that hemoglobin and platelets were increased by 13% and 44%, respectively, from baseline, and spleen and liver volumes were reduced 33% and 11%, respectively, from baseline. Changes in growth and development, bone disease, and QoL will be discussed. The majority (97.1%) of the AEs were mild or moderate, 15.7% of the AEs were reported as treatment related and one related serious AE was reported (the patient continues on treatment). There were no unexpected AEs and all treatment-related AEs were transient in nature.

Discussion: Taliglucerase alfa has the potential to provide an alternative therapy in pediatric patients with GD.

Citation

1. Pastores GM, et al. *Semin Hematol.* 2004;41:4-14.

The French Gaucher's disease registry: Clinical characteristics, complications and treatment of 562 patients.

Stirnemann J, Vigan M, Hamroun D, Heraoui D, Rossi-Semerano L, Berger MG, Rose C, Camou F, de Roux-Serratrice C, Grosbois B, Kaminsky P, Robert A, Caillaud C, Froissart R, Levade T, Masseur A, Mignot C, Sedel F, Dobbelaere D, Vanier MT, Valayanopoulos V, Fain O, Fantin B, Billette T, Mentré F, Belmatoug N.

INSERM, UMR 738, Laboratoire de biostatistiques, Hôpital Bichat, AP-HP, Paris, France.
Service de Médecine Interne, Hôpital Beaujon, AP-HP, Clichy, France.
Comité d'Evaluation du Traitement de la maladie de Gaucher.

Background: To describe clinical features, complications and treatments of patients included in the French GD registry.

Methods: All patients with known GD, living in France with ≥ 1 consultation (1980–2010) were included in the French GD Registry. We described 4 groups: 1) all registry patients (with clinical description), 2) patients with ≥ 1 visit of follow-up (to investigate occurrence of complications), 3) patients recently followed (to describe cohort of patients with good follow-up) and 4) patients treated during last 2 years (to investigate complications before and during treatment).

Data are expressed as medians (min-max range) for continuous variables and numbers (%) for categorical variables.

Results: Among the 562 registry patients (group 1): 265 (49.6%) were females; 454 (85.0%) had type 1, 22 (4.1%) type 2, 37 (6.9%) perinatal-lethal type, and 21 (3.9%) type 3. Median ages at first GD symptoms and diagnosis, respectively, were 15 (0–77) and 22 (0–84) years for all types. The first symptom diagnosing GD was splenomegaly and/or thrombocytopenia (37.6% and 26.3%, respectively). Bone-marrow aspiration and/or biopsy yielded the diagnosis for 54.7% of the patients, but enzyme deficiency was investigated to confirm the diagnosis in all patients. Birth incidence rate was estimated at 1/50 000. In the group 2, median follow-up (378 patients) was 16.2 (0.1–67.6) years. Major clinical complications were bone events [BE] (avascular necrosis, bone infarct or pathologic fracture) for 109 patients, splenectomy for 104, and Parkinson's disease for 14; 38 patients died (neurological complications for 15 type 2 and 3 type 3, complications of GD for 11 type 1 and other disease for other 9 type 1). Forty-six had monoclonal gammopathy. Among 283 patients seen recently (group 3), 36 are untreated and 247 were treated during last two years (group 4); 216 patients received treatment in December 2010 (126 with imiglucerase, 45 velaglucerase, 24 taliglucerase, 21 miglustat). BE occurred before (130 BE in 67 patients) and under treatment (60 BE in 41 patients) with estimated frequencies (95% CI) of first BE at 10 years of 20.3% (14.1%–26.5%) and 19.8% (13.5%–26.1%), respectively.

Conclusion: This registry enabled the epidemiological description of GD in France and show that BE will occur even during treatment.

Developing lentiviral vectors for gene therapy of type I Gaucher disease.

Aitchison KL, Rahim AA, Kinnon C, Hughes D, Waddington SN, Howe SJ.

Background: Gene therapy has been used to treat a number of diseases of haematopoietic origin including immunodeficiencies and leukodystrophies. However gene therapy for Gaucher Disease (GD) has yet to be realised. Type I GD is effectively treated by enzyme replacement therapy but this treatment option has to be repeated every two weeks throughout the life of the patient and does not always effectively treat the skeletal complications. Gene correction and transplantation of autologous haematopoietic stem cells could provide a method of treating Type I GD which overcomes these barriers.

Methods: We have designed a number of lentiviral vectors carrying the glucocerebrosidase (GBA) gene and are now in the process of testing and comparing these vectors. We have produced two versions of the GBA gene which has been fused with the protein transduction domain of the HIV-1 TAT protein in order to provide a method of uptake for GBA secreted by the transduced cells that is independent of the mannose receptor pathway.

Results: We show that all the vectors express GBA enzyme by means of functional assays and Western blot. We also demonstrate that when human embryonic kidney cells are transduced with a multiplicity of infection (MOI) of 100 active protein is secreted ($p < 0.005$) and that this secreted enzyme can be used to correct untransduced patient cells in a co-culture system (<50% increase in GBA positive cells after exposure to supernatant). This presents an opportunity for the cross correction of cells in tissues other than the haematopoietic system if used *in vivo*.

Conclusions: Gene therapy could be an effective and efficient way to treat Type I GD and we have shown that lentiviral vectors can be used to treat not only the directly transduced cells but also the neighbouring cells of other systems. In the future we plan to test these vectors further using a range of cell types and also material from GBA-deficient mice. Further tests aim to differentiate corrected haematopoietic stem cells into osteoclasts to investigate bone pathology and gene therapy as an avenue to treat the skeletal complications of Type I GD.

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Poster 01

Variability assessment of spleen volume measurements based on MRI sequence selection in Gaucher subjects.

Bracoud L¹, Ahmad H¹, Lopez A¹, Brill Almon E², Chertkoff R.

(1) BioClinica Inc. (2) Protalix Biotherapeutics

One characteristic clinical finding of Gaucher disease is massive splenomegaly. In order to accurately assess splenic volume and its longitudinal changes, it is important to minimize measurement variability which may result from the measurement technique itself, MRI acquisition or potentially endogenous factors (metabolism, time since last meal, etc.). Systematically using the same MRI sequence to perform the measurements with consistent MRI parameters and optimal image quality can alleviate variability. However, in situations where that sequence is impaired by significant image artifacts, using another available MRI sequence to perform the measurement would be beneficial.

The aim of this study is to assess the potential impact on measurement variability when various MRI sequences are used for assessment of spleen volume in Gaucher subjects.

Baseline imaging data with no significant image artifacts from 10 treatment naïve Gaucher clinical trial subjects were selected with >8 MN splenomegaly. Transverse T2 and In/Out-of-phase (6 mm slices with 1 mm gap), transverse T1 and coronal T1 sequences (5 mm slices from a 3D acquisition) were available for all subjects. Spleen volume was semi-automatically delineated on each sequence using a 3D automatic unsupervised bayesian segmentation followed by manual correction by 2 experienced technologists. Inter-reader variability was assessed for each sequence. T2 was then considered as the reference sequence while variability with the other sequences was assessed.

Mean inter-reader variability (resp. standard error) was 1.4% (0.2%) for the T2 sequence, 1.7% (0.2%) for the transverse T1, 0.4% (0.1%) for the coronal T1, 1.0% (0.2%) for the In-phase and 1.4% (0.2%) for the Out-of-phase. Absolute variability (resp. standard error) compared to the measure done on T2 was worth 4.7% (1.0%) for the transverse T1, 3.6% (0.8%) for the coronal T1, 8.0% (1.4%) for the In-phase and 4.3% (1.1%) for the Out-of-phase.

Inter-reader variability was very low (compared to the usual amount of change expected to be reached during the course of a study to prove efficacy, e.g. >20%) for all sequences. When comparing T2 measurements with those done on other sequences, the In-phase echo seemed to overestimate the volume compared to the others. Considering inter-reader variability and expected amount of change, variability was low enough to allow the use of any other sequence when needed to assess longitudinal changes.

Poster 02

Longterm outcome of type III Gaucher disease children.

Arends M, Biegstraaten M, Aerts JM, Wijburg FA, Hollak CE.

Department of Internal Medicine, division Endocrinology & Metabolism, Academic Medical Center, the Netherlands

Introduction : Type 3 Gaucher disease (GD3) is characterized by hepatosplenomegaly, cytopenia and skeletal disease as well as central nervous system involvement with variable severity. Treatment of GD3 consists of enzyme replacement therapy, which ameliorates the systemic symptomatology but does not influence the neurological symptoms. Miglustat, a glucosylceramide synthase inhibitor, partially crosses the blood brain barrier and has proven to have some effect on systemic symptoms. Effects of miglustat on the brain are still a matter of debate.

Aim : The objective of this study is to describe the effects of miglustat on the neurological signs and symptoms in Dutch GD3 patients

Methods : A retrospective study is performed by reviewing the charts of the GD3 patients in the Netherlands. The modified severity scoring tool (mSST) is used as primary outcome measure. In addition, data on other neurological symptoms, evoked potentials, EEGs and eye movement tests as well as chitotriosidase, CCL18/PARC, haemoglobin levels, platelet counts, and radiology are collected. Baseline is defined as the day of start of miglustat treatment.

Results : Six patients with GD3 (median age 11.5, range 0.4 – 17.4) have been followed at the AMC with a median follow-up time of 4.4 years (range 1 – 6.9 years). All patients were treated with miglustat, starting at a median of 9.8 years (range 0.3 – 13.7) after GD diagnosis and 9.5 years (range 0 – 13.4) after introduction of ERT. One patient switched from ERT to miglustat after 8.8 years. Mean increase in mSST was 0.3 points per year (range 0 – 0.57). Systemic disease remained stable or improved in all but one patient, who was treated with miglustat monotherapy and showed a more than six fold increase in chitotriosidase.

Discussion : Despite treatment with miglustat progression of neurological disease was seen. A larger collaborative study is needed to analyze whether miglustat slows down the rate of disease progression as compared to monotherapy with ERT

Poster 03

Focal Segmental Glomerulosclerosis (FSGS) in Type 1 Gaucher Disease.

Marega A¹, Bembi B², Sechi A², Ciana G²; Pizzolitto S³, Romano G¹, Gropuzzo M¹, Vallone C¹, Montanaro D¹.

¹ Department Specialistic Nephrology; ² Department Laboratory Med Rare Disease;

³ Department Lab Diagnostic Pathology; AOUD Udine Italy.

Introduction : Gaucher disease (GD) is a genetic lysosomal disease characterized by deficiency in glucocerebrosidase. This abnormality induces the formation of Gaucher cells (GC) that infiltrate bone marrow, spleen, liver, lungs² and brain and are considered to be mainly responsible for the clinical manifestations of GD¹. Despite the multiorgan nature of the disease a systematic evaluation in patients with GD usually don't evidence renal abnormalities³.

Aim : We describe a glomerulonephritis in a patient with GD.

Methods : A 55 years old Caucasian man was diagnosed GD type 1 N3705S. Because of progressive thrombocytopenia and splenomegaly he underwent splenectomy at age 56. GD had been diagnosed previously in his older two sisters, who had developed Parkinson disease. At age 72, the patient was diagnosed JAK2 positive myeloproliferative disorder (MPD). In the same year he underwent prostate resection for hypertrophy. In 2009, at age 75, the patient was discovered heavy proteinuric (6 gr/die), with serum creatinine 1 mg/dl; it was started ramipril as antiproteinuric agent and oncocarbide for MPD. In 2011, with proteinuria 8 gr/die, a renal biopsy was performed. Of 31 glomeruli, 3 had global sclerosis, while the other showed focal segmental sclerosis of the glomerular tuft with synechiae to Bowman's capsule and hyalinosis involving foam cell. Interstitial compartment showed patchy tubular atrophy and focal fibrosis. The interlobular arteries showed mild atherosclerosis. Immunofluorescence showed focal segmental positivity for IgM (+). The diagnosis was FSGS. Months later the proteinuria decreased spontaneously (0.4 gr/die). For severe bone pain the patient was recovered. He deceased by cardiopulmonary arrest. The autopsy revealed acute myocardial infarction and lung adenocarcinoma.

Results : FSGS can be associated to GD.

Discussion : GD with severe clinical and pathological renal involvement is exceptionally rare⁴. In literature there are only few cases in which the renal involvement is characterized by heavy proteinuria and/or renal failure caused by the accumulation of GC in glomeruli and interstizium of the cortex; in another report a boy was diagnosed GD and mesangiocapillary glomerulonephritis⁵. Here we have described a patient with GD and heavy proteinuria secondary to FSGS. The proteinuria spontaneously decreased. To our knowledge this is the first combination of FSGS and GD and the second case of JAK2 MDP and GD in the literature.

Citations : ¹ Berger J, British Journal of hematology, 2010; 150, 93-101.

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⁵ Halevi R, Pediatr Nephrol, 1993; 7, 438-440

Poster 04

Screening for Gaucher disease in patients with splenomegaly of unknown origin: results from a nation-wide pilot study in 167 patients.

Herrmann A, Bange M, vom Dahl S.

Dept. of Internal Medicine/Gastroenterology, St. Franziskus-Hospital,
Academic Teaching Hospital, University of Cologne, Schoensteinstrasse 63, 50825
Cologne, Germany

Introduction: There is a discrepancy between predicted (2000) and identified Gaucher patients in Germany (≤ 300 patients). Splenomegaly can be caused by a) vascular changes, b) hyperplasia of red or white spleen pulp, c) storage material, d) infiltration by tumour cells, e) fibrosis or f) a combination of these factors. Patients with splenomegaly of unknown origin are usually referred to hematooncologists for the medical workup. Identification of GD is sometimes missed, because bone marrow smears are not being done, not analyzed properly or are devoid of typical Gaucher cells. After exclusion of hepatological, malignant, haematological or infectious causes of splenomegaly, the presence of lysosomal storage disease could be suspected.

Aim: The leucocyte activity of b-glucocerebrosidase (GBA) and plasma chitotriosidase (CT) in patients, in whom liver cirrhosis, haematological disease or malignancy or an infectious cause of splenomegaly were largely excluded, was determined in a nation-wide screening project with hematologists/oncologists.

Methods: Hematooncologists, either in practice or in hospital settings, were offered determination of GBA and CT activity in patients with splenomegaly of unknown origin. Together with the sample, a questionnaire asked for anonymized patient data (year of birth, gender, presence and degree of splenomegaly and/or hepatomegaly, presence of thrombocytopenia and/or anemia, increase of plasma ACE activity, presence of hyperferritinemia, presence of bleeding symptoms, bone pain and osteonecroses) and for the hitherto performed differential diagnostics (lymphoma, leukemia, multiple myeloma, infection excluded). The study was performed between 2008 and 2011. Analyses were performed by a certified laboratory (Laboratory Molec. Genet. Metab. Shin-Podskarbi, Munich, Germany).

Results: Between 2008 and 2011, 167 samples were submitted for GBA and CT analysis. Sixty percent were male, the patients' age ranged from 1-100 years. Splenomegaly was present in 83 % of the patients, classified as being severe in 34 % of the patients. Hepatomegaly was present in 44 %, with 7 % being classified as being severe. Thrombocytopenia was present in 58 % of the patients, whereas 44 % were classified as being anemic. High serum ferritin was present in 17 % of the cohort, and increased serum ACE activity was found in 4 % of the patients. Bone pain was reported in 43 % of the patients, with only 7 % exhibiting osteonecroses. Bleeding tendency was reported in 14 % of the patients. Lymphoma had been excluded in 76 %, leukemia excluded in 75 % and multiple myeloma had been excluded in 70 % of the cohort. Finally, in 67 % of the patients, an infection had been excluded. The lab results showed increased plasma CT activities in 18/167 patients. Out of 167 patients, six patients referred by hematooncologists had pathological GBA activities. Out of these six patients, the age ranged from 1-76 years. Clinical data are evaluated and the pathological GBA and CT activities are currently remeasured.

Discussion: In a cohort of mostly splenomegalic patients, referred by hematooncologists, 10 % seemed to display pathological CT activities, about 3-4 % had significantly decreased GBA activities. Pending a confirmation of the results, a prevalence of GD in 1: 30

idiopathic splenomegalies is possible. The even higher prevalence of high CT activities in this cohort mandates a diligent search for other diseases associated with splenomegaly and high CT activities, e.g. Niemann-Pick diseases, cholesterol ester storage diseases and thalassemia. The results have to be seen with caution, since a revalidation of pathological lab findings has not been finalized. Due to the anonymized nature of the screening study, a validation of pathological results is currently under way, together with a post-hoc workup on clinical parameters of those patients presenting pathological results. If the results are to be confirmed, idiopathic splenomegaly is the clinical finding to be focussed at to identify Gaucher patients.

Address of authors: St. Franziskus-Hospital Köln, Academic Teaching Hospital of the University of Cologne, Schönsteinstrasse 63, 50825 Köln, Telefon: +49-221-5591-1101, Telefax: +49-221-5591-1103, E-Mail: stephan.vomdahl@st-franziskus-koeln.de.

Grants: This study was supported by a restricted research grant from Genzyme, Neu-Isenburg, Germany.

Poster 05

Evaluation of Taliglucerase alfa efficacy respective to therapeutic goals.

Pastores G¹, Fletcher N², Silverstone J³, Messig M³, Paz A⁴, Brill-Almon E⁴, Golembo M⁴, Chertkoff R⁴.

¹New York University School of Medicine, Neurogenetics Unit, New York, NY, USA; ²Pfizer, Sydney, NSW, Australia; ³Pfizer, New York, NY, USA; ⁴Protalix Biotherapeutics, Carmiel, Israel.

Introduction: Therapeutic goals set forth by an international panel of expert Gaucher disease (GD) clinicians have been used to benchmark enzyme replacement therapy (ERT) efficacy for type 1 GD.¹ Taliglucerase alfa is a plant-cell-expressed United States Food and Drug Administration–approved ERT for GD.

Aim: The purpose of this analysis was to assess the achievement of therapeutic goals for 4 clinical parameters through 24 months of treatment with taliglucerase alfa.

Methods: This is a post hoc analysis based on the taliglucerase alfa phase 3 randomized, double-blind study² for the first 9 months and blinded extension for months 12 and 24. Inclusion criteria specified spleen volume ≥ 8 multiples of normal (MN) and thrombocytopenia of $< 120,000$ platelets/mm³. Patients were randomized to taliglucerase alfa 30 or 60 U/kg. This analysis includes only the patients who were treated with taliglucerase alfa 60 U/kg; patients with treatment interruptions > 4 weeks were excluded.

Results: Of the 26 patients with 24-month data, 10 patients received taliglucerase alfa 60 U/kg and had no treatment interruptions > 4 weeks. At 9, 12, and 24 months, respectively, 87%, 100%, and 70% of patients achieved the therapeutic goals for spleen volume and 60%, 89%, and 78% achieved the goals for platelets. Therapeutic goals for liver volume and hemoglobin were achieved in 100% of patients at months 12 and 24. At 24 months, 70% of patients achieved all 4 therapeutic goals.

Discussion: In this post hoc analysis study of patients with type 1 Gaucher disease, ERT with taliglucerase alfa 60 U/kg effectively improved disease, as assessed by achievement of therapeutic goals by months 12 and 24 for splenomegaly, hepatomegaly, thrombocytopenia, and anemia.

Citations

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2. Zimran A, et al. *Blood.* 2011;118(22):5767-5773.

Poster 06

Severe bone involvement in Gaucher patient: Tremendous results of combined treatment.

Lukina K, Mamonov V, Sysoeva E, Pisetsky M, Yatsyk G, Lukina E.

Hematology Research Center of Ministry of Healthcare and Social Development of the Russian Federation Moscow, Russia.

Case report: Hepatosplenomegaly was revealed in 1987 at the age of 1,5 yrs. Gaucher disease (GD) was diagnosed and splenectomy was performed. During the following 15 yrs the patient lived a normal life.

From 2001 (15 yrs old) till 2006 the patient suffered 11 pathological fractures:

2001 – fracture of lower 1/3 of left femur, lower 1/3 and middle 1/3 of left shin.

2004 – fractures of both femurs. Since 2004 the patient was bedridden and could move only with the help of a wheelchair.

2006 – fractures of both humeri and both femurs.

The patient did not receive enzyme replacement therapy (ERT) as it was not available at that time.

January 2009 – first admission to our Center. On admission: the patient was bedridden and needed constant relatives' care. Huge hepatomegaly was present (+4 cm below umbilical ring), right thigh was enlarged (the diameter was 57 cm), the patient had false right elbow joint so the support function of right hand was absent. The diagnosis of GD was confirmed by enzyme assay. Laboratory tests revealed markers of chronic hepatitis C and inherited thrombophilia (prothrombin mutation). The diagnosis was established: GD type I with severe bone involvement. Splenectomy. Chronic viral hepatitis C (HCV-RNA -, AST/ALT=N). Inherited thrombophilia.

Treatment included:

- imiglucerase 60 U/kg/infusion
- surgery – intramedullary osteosynthesis of right humerus
- metabolic treatment: bisphosphonates, vitamins
- physical training (when the function of right hand was restored, the patient was gradually trained to stand with support)

The patient was discharged from our Center after 4 months of hospitalization (June 2009). She could already stand with the help of crutches and walk for short distances.

At home the patient continued to receive ERT. Also the patient constantly trained to walk.

Next control examination in our Center was in June 2010. We observed marked positive effect of the treatment: liver volume decreased significantly, the patient could walk with the help of crutches for long distances.

October 2011 – next control examination: the patient was feeling well and was able to walk without orthopedic aid.

Discussion: this case demonstrates extremely severe bone involvement in Gaucher patient with numerous pathological fractures and long period of immobilization. The tremendous result was achieved owing to ERT, surgical treatment and special methods of activation: the patient is able to walk without orthopedic aid and leads a normal active life.

Poster 07

Evaluation of bone mineral density and elasticity in type 1 Gaucher disease : effect of Miglustat therapy.

Roca M¹, Civeira I², Latre P³, Alfonso P^{1,4,5}, Irun MP^{1,4}, Gervas J^{1,5}, Pocovi M^{1,3,4,6}, Giraldo P^{1,3,4,5,6}.

¹Instituto Aragonés Ciencias de la Salud, ²Centro de Investigación de Osteoporosis (CEINOS), Zaragoza, Spain ³FEETEG ⁴Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER)⁵Traslational Research Unit. Miguel Servet University Hospital. Zaragoza. Spain. ⁶Hematology Department. Miguel Servet University Hospital. Zaragoza. Spain. ⁷Department of Biochemistry and Cellular and Molecular Biology, University of Zaragoza

Background: Bone disease is the most serious manifestation of type 1 Gaucher disease (GD1). Long-term enzyme replacement therapy (ERT) has only limited efficacy on skeletal problems in patients with Gaucher disease. In preliminary studies miglustat has shown a positive effect in bone mineral density (BMD). The standard technique for evaluation of BMD is dual-energy X-ray absorptiometry (DEXA), which measures bone density in the axial skeleton. There are methods of examining bone density, however, that use ultrasound instead of X-rays, that are able to measure the amount of mechanical energy transmitted by the bone, as well as their speed of transmission (broadband ultrasound attenuation; BUA).

Aim: To compare ultrasound techniques with DEXA in the evaluation of BMD and elasticity in patients with GD1, as well as the effect of therapy with miglustat on bone density.

Methods/patients: Data from 93 GD1 adult patients, stratified by age and gender, were analysed. Bone marrow changes were evaluated by a semi-quantitative score (Spanish-Magnetic Resonance Imaging [S-MRI]. Bone density and BUA was evaluated by using calcaneal ultrasound CUBA CLINICAL BONE DENSITOMETER (Norland Medical Systems). In addition, for each individual, the genotype, clinical characteristics and profile of surrogate biomarkers, S-MRI and response to therapy was analysed. Results: The 76% of GD1 patients had decreased bone density (55% osteopenia and 21% osteoporosis according WHO criteria) at diagnosis. The effect of treatment with Miglustat on BMD was evaluated in 24 patients. After 24 months on miglustat therapy the mean change in Z-score was 0.09, 0.42 in right calcaneous and 0.09, 0.30 in left calcaneous (p=0.01)

Conclusions: Miglustat-induced improvement in the BUA may reflect more effective anti-inflammatory effect related to remodeling bone structure. Nevertheless, more patients and more time of follow-up are necessary to confirm these results.

Poster 08

MR Imaging of skeletal complications in paediatric patients with Gaucher disease type I and effect of Enzyme Replacement Therapy (ERT).

Anastasiou A¹, Vasimidou K¹, Barmpas N¹, Vargiami E², Liatopoulos G¹, Kyriakou D¹, Notidis A¹, Papadopoulos S¹, Zafeiriou D².

¹ Department of Radiology, «Hippokratio» General Hospital, Thessaloniki, Greece,

² 1st Department of Pediatrics, Aristotle University, Thessaloniki, Greece.

Introduction/Aim: Gaucher's disease (GD) is an inherited metabolic disease. It is caused by a deficiency of the lysosomal enzyme glucocerebrosidase, which leads to the pathologic accumulation of the enzyme substrate glucocerebroside, within the macrophages of the reticuloendothelial system. The objective is to present the anatomical sites of skeletal involvement in a series of five paediatric patients with GD type I and the improvements with Enzyme Replacement Therapy (ERT) during the last 10 years.

Methods: MRI images were obtained using a 1.5-T magnet (Symphony; Siemens Erlangen, Germany). We performed the study on 5 patients (1 boy and 4 girls) with type I GD, whose ages ranged from 2,5 years to 14 years. A series of contiguous coronal images was made, supplemented by sagittal or transverse projections, or both using T1/T2/STIR-weighted sequences of the peripheral and axial skeleton including the entire tibiae.

Results: Two of the patients demonstrated a heterogeneous pattern of bone marrow infiltration in both femora and tibiae and 1 patient on both tibiae and epiphyses. One patient demonstrated a heterogeneous pattern of infiltration of both femora, tibiae and epiphyses and bone infarcts in both femora and tibiae. The fifth patient demonstrated Erlenmeyer flask deformity. The total follow up period ranged from 4 to 10 years. Skeletal improvements with ERT were observed in 4 patients and stabilization in 1.

Discussion: The skeletal complications of GD are progressive and MRI is the most applicable imaging technique for detection and evaluation of bone marrow infiltration. We classify our patients according to the system developed at the Institute of Diagnostic Radiology, Dusseldorf. In GD the fat marrow is replaced by the infiltration of Gaucher cells and is best detected with low signal intensity on T1/T2 weighted images. GD is present initially in the lumbar spine but with progression of disease the extremities become more affected. The epiphyses are spared except in the most severe cases, so there is a typical centrifugal spread of bone marrow infiltration in the disease progression. At birth, bone marrow is principally red and converted to yellow in a centripetal direction, whereas epiphyses contain yellow marrow. These changes in childhood and adolescence can be difficult to distinguish by MRI from Gaucher cell infiltration and the best way to detect severe disease in these patients is to perform MRI of the tibia as we did in all our 5 patients.

Poster 09

Assessment of peri-prosthetic bone density in patients with Gaucher disease after total hip arthroplasty.

Bregman A, Elstein D*, Tiomkin M*, Zimran A*, Lebel E.

Department of Orthopedic Surgery and Gaucher Clinic*, Shaare Zedek Medical Center, affiliated with the Hebrew University School of Medicine, Jerusalem Israel.

Introduction: Skeletal involvement, i.e., osteopenia, osteonecrosis, and bone pain, is still considered an unmet need of Gaucher disease. Total hip arthroplasty (THA) is performed for painful hip-joint arthropathy due to femoral head osteonecrosis and joint degeneration. Concerns of poor osteo-integration of metal prosthetic stems in Gaucher disease were rejected after short- and intermediate-term outcome studies showed good results. Dual energy X-ray absorptiometry (DEXA) is a quantitative method for assessment of bone mineral density (BMD) that may serve for assessment of bone integration of hip joint implants. DEXA allows evaluation of peri-prosthetic density changes reflecting appropriate bone in-growth or loosening of the prosthesis.

Aim: Evaluation of peri-prosthetic BMD around femoral stems after cement-less THA. Findings are compared to peri-prosthetic BMD in non-Gaucher with THA and to disease Gaucher severity factors and a functional hip score.

Methods: All patients available for THA assessment and routinely followed in our Gaucher Clinic were invited to evaluate hip implants along with annual DEXA. Assessment of peri-prosthetic BMD was performed with specific software for Hologic® DEXA. Results were calculated as BMD for each of 7 Gruen-zones around the femoral prosthetic stem and total peri-prosthetic density. Background medical data was collected and hip joint function estimated by Harris Hip Score (HHS).

Results: There were 21 Gaucher patients (8 females; mean age 57 [range 30-81] years, all receiving enzyme replacement therapy [ERT]) with 22 prosthetic THA stems. "Prosthesis age" range: 1-19 years (mean: 9 years). Excluding 1 patient with established femoral stem loosening (18 years post-op), all patients had DEXA results comparable to published data of BMD adjacent to cement-less femoral stems in non-Gaucher patients. These results support normal bone in-growth in Gaucher disease. Correlation studies did not reveal worse regional BMD in compound heterozygotes, shorter ERT duration, or less good HHS scores. There was a negative correlation between density levels and BMI, possibly a testing artifact.

Discussion: We present a low-cost, practical modality for evaluation of hip prostheses which we recommend be integrated into annual DEXA of patients with hip joint implants. Our preliminary report shows normal BMD around cement-less femoral stems, supporting earlier studies that THA is appropriate for degenerative hip disease in Gaucher disease.

Poster 10

Bone mineral density response to enzyme replacement therapy over 2 years in adults with type 1 Gaucher disease.

Zimran A^a, Kabra M^b, Lukina EA^c, Giraldo P^d, Kisinovsky I^e, Bavdekar A^f, Ben Dridi MF^g, Kishnani PS^h, Mehta Aⁱ, Elstein D^a, Wang N^j, Hangartner TN^k, Crombez E^j, Ben Turkia H^g.

^aShaare Zedek Medical Center and Hebrew University-Hadassah Medical School, Jerusalem, Israel; ^bAll India Institute of Medical Sciences, New Delhi, India; ^cHematology Research Center, Moscow, Russia; ^dCIBERER and Hospital Universitario Miguel Servet, Zaragoza, Spain; ^eYour Health SA, Buenos Aires, Argentina; ^fKEM Hospital Research Centre, Pune, India; ^gLa Rabta Hospital, Tunis, Tunisia; ^hDuke University Medical Center, Durham, NC, USA; ⁱRoyal Free Hospital, London, UK; ^jShire Human Genetic Therapies, Inc., Lexington, MA, USA; ^kWright State University, Dayton, OH, USA.

Introduction : Improving bone mineral density (BMD) is a therapeutic goal in the treatment of type 1 Gaucher disease (GD1).

Aim : To assess the BMD response to enzyme replacement therapy in GD1 adults from trial HGT-GCB-039 and the extension study HGT-GCB-044.

Methods : GD1 patients were allocated to 60 U/kg every other week (EOW) of either velaglucerase alfa (velaglucerase alfa arm) or imiglucerase (imiglucerase/switch arm) in HGT-GCB-039 (9 months), followed by 15 months of velaglucerase alfa 60 U/kg EOW (both arms) in HGT-GCB-044. To measure BMD in adults, dual-energy x-ray absorptiometry of the lumbar spine (LS) and femoral neck (FN) was used. BMD T-scores and age- and sex-matched Z-scores were calculated.

Results : Excluding patients on concomitant bisphosphonates (BPs), 11 velaglucerase alfa patients (median age: 38 [range: 19–58] years; sex 7 F, 4 M) and 8 imiglucerase patients (median age: 29.5 [range: 20–44] years; 7 F, 1 M) were studied: median (range) LS Z-scores at Baseline were -1.46 (-3.50–0.98) and -0.86 (-2.17–2.02), respectively. In the velaglucerase alfa arm, the mean (95% CI) LS Z-score change from Baseline was 0.33 (0.10, 0.55) at 9 months and 0.64 (0.22, 1.06) at 24 months; in the imiglucerase/switch arm, it was 0.06 (-0.22, 0.34) at 9 months and 0.54 (0.21, 0.87) at 24 months (similar means if patients on BPs—2 velaglucerase alfa [age in years/sex: 18/M and 60/F] and 3 imiglucerase/switch [35/F, 48/M and 58/M]—included). Mean FN Z-score changes from Baseline were not significant ($P > 0.05$; both arms, \pm BPs). Velaglucerase alfa patients classified per the WHO T-score criteria as osteoporotic, osteopenic or normal numbered 2, 7 and 2 for the LS and 0, 6 and 5 for the FN at Baseline, then 1, 4 and 6 for the LS and 1, 6 and 4 for the FN at 24 months. At Baseline, all 8 imiglucerase/switch patients had normal FN BMD whereas the LS was osteoporotic, osteopenic and normal in 1, 3 and 4, respectively; no change was seen at 24 months (either site).

Discussion : 9 months of velaglucerase alfa resulted in significantly increased LS BMD Z-scores in GD1 adults, which was not achieved in the imiglucerase-treated patients. In the subsequent 15 months of velaglucerase alfa, Z-scores increased further in patients previously exposed to velaglucerase alfa, and a statistically significant improvement was reached in those who switched from imiglucerase. Shifts across clinically significant BMD classifications were seen in the LS by 24 months of continuous velaglucerase alfa.

Poster 11

Comparative efficacy analysis of published literature of Taliglucerase alfa with Imiglucerase and Velaglucerase alfa.

Becker P¹, Weinreb N², Messig M³, Marsden D³.

¹Department of Medicine/Division of Hematology, Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle, WA, USA; ²University Foundation for Lysosomal Storage Disorders, Coral Springs, FL, USA; ³Pfizer, New York, NY, USA

Introduction: Taliglucerase alfa is a plant-cell-expressed enzyme replacement therapy (ERT) recently approved for Gaucher disease (GD) in the United States. There have been no randomized clinical trials directly comparing taliglucerase alfa to other approved ERTs, but appreciable data in 6 high quality studies of either imiglucerase or velaglucerase alfa offer are available for retrospective analysis.

Aim: To provide contextual evaluation of taliglucerase alfa efficacy results compared with documented effects of imiglucerase and velaglucerase alfa in similar patient populations.

Methods: Electronic searches of the MEDLINE database from 1994–May 2011 were performed and abstracts from major genetic and metabolic conferences from March 2010 to March 2011 were reviewed. Two independent reviewers applied the following criteria for inclusion of studies: treatment-naïve adults; intact spleen; treatment with placebo or 30 to 60 U/kg ERT to 12 months; clinical endpoints of spleen volume, liver volume, hemoglobin, and platelet count. Study quality was assessed and rated by 2 independent reviewers using a modified Downs and Black checklist with a maximum score of 16; studies with scores over 10 were considered good. Estimates of the mean and standard deviation were extracted from each study (or calculated from individual response data).

Results: Five journal publications and 1 congress abstract on imiglucerase or velaglucerase alfa met the inclusion criteria, received quality scores ranging from 11 to 16, and were compared against 1 taliglucerase alfa publication. Mean values for the baseline endpoints of spleen volume, liver volume, hemoglobin, and platelet count were similar for the studies included. The standard deviations for the mean change from baseline in spleen volume, liver volume, hemoglobin, and platelet count for taliglucerase alfa (30/60 U/kg) showed overlap with that of velaglucerase alfa (45/60 U/kg) and/or imiglucerase (60 U/kg) at months 6, 9, and 12. Efficacy endpoint data for both dose groups of taliglucerase alfa fell within the range of data from the International Collaborative Gaucher Group Registry. Subgroup analysis of patients stratified by baseline severity of splenomegaly, anemia, or thrombocytopenia was confirmatory of these findings.

Discussion: The results of this analysis support the conclusion that the magnitude of effect size is comparable between taliglucerase alfa, imiglucerase, and velaglucerase alfa.

Poster 12

Safety of use of velaglucerase in four patients with type 1 Gaucher's disease shifted from imiglucerase.

Ciana G, Sechi A.L, Dardis A, Deroma L, Macor D, Bembi B.

Regional Coordinator Centre for Rare Diseases, University Hospital "Santa Maria della Misericordia", Udine, Italy.

Background: The shortage of beta glucosidase in years 2009-10 caused a reduction in supplying the drug to Gaucher type 1 patients. In our casuistry the reduction was well tolerated in most of the patients, while few suffered of this shortage with a symptomatology mainly dominated by asthenia and bone pain .

Patients and methods : In four of the latter patients , it was possible to shift from beta glucosidase to velaglucerase, which was given at the dosage preceding the shortage (ranging from 60 to 94U/Kg/month)

Results: After one year of follow up, an improvement of asthenia was documented in all the patients, bone pain improved in 3 patients . Haemoglobin and platelets levels did not show significant variations from the time of the shift . Liver and spleen volume did not change significantly. MR of femurs did not show signification worsening of infiltration where present, while DEXA z score , measured al lumbar level, remained on the same values. No side effects were recorded.

Conclusions: Velaglucerase (Vpriv) showed to be safe and able as beta glucosidase in maintaining stable the main indicators of disease burden.

Poster 13

PARC during the shortage of Cerezyme.

Mengel E.

Introduction : Chitotriosidase and PARC are biomarkers of Gaucher disease. We investigate PARC and Chitotriosidase in 2 different patient groups before and after shortage related dose reduction and controls.

Methods: We compared 6 groups of 20 patients each. Patients with a stabil clinical picture were observed before the shortage of enzyme (group 1) and afterwards (group 2). Group 3 and 4 consists of patients with deterioration during shortage, again before (group 3) and after shortage period of enzyme replacement (group 4). Group 5 contains patients naïve to enzyme replacement therapy (ERT) yet. Group 6 contains healthy persons and serves as our control group.

Results: The PARC median of group 1 is 143 µg/ml and of group 2 145,5 µg/ml. The median of group 3 is 197 µg/ml and of group 4 211,5 µg/ml. The median of group 5 is 790 µg/ml and of group 6 31 µg/ml. For Chitotriosidase the following medians were detected: group 1 621,48 mg/ml/h, group 2 903,5 mg/ml/h, group 3 843 mg/ml/h, group 4 1267,5 mg/ml/h, group 5 17922,5 mg/ml/h, group 6 67 mg/ml/h.

Discussion: Both biomarkers demonstrate the same attitude. Group 5 with non ERT has the highest and, the control group (group 6) the lowest value. Values of solid patients (group 1 & 2) are significantly lower than those of insecure patients (group 3 & 4). Biomarkers are therefore dependent of the clinical picture. The median PARC after the the shortage of enzyme is almost the same as before the shortage for solid patients (group 1 and 2), but there is a significant difference for patients with an insecure clinical picture (group 3 and 4).

Conclusion: The clinical course during the therapy-free period depends on the initial clinical situation. There is a significant rise of median PARC after the therapy-free period only for insecure patients.

Poster 14

GBA1 Mutations and genotype-phenotype correlation in patients with Gaucher disease type 1 from the Stockholm area.

Machaczka M¹, Paucar Arce M², Bulanda A¹, Engvall M³, Pettersson A¹, Hägglund H¹, Svenningsson P².

¹Department of Medicine at Huddinge, ²Department of Neurology and Department of Clinical Neuroscience, and ³Centre for Inherited Metabolic Diseases, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden.

Introduction: In Sweden, with a population of approx. 9.4 million people, the overall prevalence of Gaucher disease (GD) is approx. 1:170,000 inhabitants. This number is a little lower than the GD prevalence reported in other Western countries but is 2.5 times higher than in other Nordic countries. Although the Norrbottnian form of GD is a unique form of GD type 3 (GD3) that is typical for ethnic Swedish patients (pts) from Northern Sweden, currently the majority of Swedish GD pts (64%, 35/55) have the GD type 1 (GD1) phenotype. So far, there was no published data on the prevalence of *GBA1* mutations in Swedish pts with GD1.

Aim: To present the prevalence of *GBA1* mutations and GD1 phenotypes in pts from the Stockholm area.

Methods: Between 2002 and 2011, 13 pts were diagnosed with GD1 in the Stockholm area. In all studied pts, the diagnosis of GD was confirmed by a low activity of β -glucosidase in peripheral blood leukocytes and increased activity of plasma chitotriosidase. Further direct DNA sequencing revealed mutations in the *GBA1* gene in all cases. The files were reviewed for collection of relevant clinical data.

Results: All but one patient (92% of GD1 pts) carried at least one allele with a c.1226A>G (N370S) mutation in the *GBA1* gene. Interestingly, all ethnic Swedish pts (7/7) were heterozygous for one allele with a potentially neuronopathic mutation (i.e., leading to the neuronopathic GD phenotype when homozygous), and presented as follows: c.1448T>C (L444P) in 3 pts, and c.437C>T (S107L), c.721G>A (G202R), RecNci I, c.330delA in the remaining 4 pts, respectively. The median age of the ethnic Swedish pts at diagnosis was 30 years (range 3–61 years); three of them were splenectomized and two of them developed Parkinson's disease. The remaining 6 pts with GD1 included 3 pts who were homozygous for c.1226A>G (N370S), 2 pts who carried one allele with c.1226A>G (N370S) and a second allele consisting of c.115+1G>A (IVS2+1) or RecNci I, respectively, and one patient who was heterozygous for the novel mutations c.798C>G and c.1040T>G. The median age of the non-ethnic Swedish GD1 pts at diagnosis was 53 years (range 3–60 years); only one of them with the most severe phenotype and heterozygous private *GBA1* mutations was splenectomized in Iran at the age of 5 years. None of the non-ethnic Swedish GD1 pts developed Parkinson's disease.

Discussion: These results suggest that ethnic Swedish GD1 patients are more frequently heterozygous for the potentially neuronopathic mutations when compared with other GD1 patients. It is possible that ethnic Swedish GD1 patients experience a more severe GD1 phenotype and have a somewhat higher risk of developing Parkinson's disease. Certainly, our results must be interpreted with caution due to the small number of analyzed patients.

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Poster 15

Atypical morphology of Gaucher cells is frequently seen in bone marrow smears from untreated patients with Gaucher disease type 1 – A cytological study.

Machaczka M¹, Regenthal S², Bulanda A¹, Galazka K³, Kleinotiene G⁴, Hägglund H¹, Klimkowska M².

¹Department of Medicine and ²Department of Clinical Pathology and Cytology, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden; ³Department of Pathology, Collegium Medicum of the Jagiellonian University, Cracow, Poland; ⁴Centre of Hematology and Oncology, Children's Hospital, University Hospital Santariskiu, Vilnius, Lithuania.

Introduction: Gaucher cells (GCs), the lipid-laden storage macrophages, are the pathologic hallmark of Gaucher disease (GD). GCs are typically 20-100 µm in diameter with eccentrically placed nuclei and cytoplasm with characteristic crinkles and striations (i.e., so-called crumpled/wrinkled tissue appearance) (Figure, a). However, only a few previous observations have indicated that sometimes GD patients may display morphology of GCs which is different from this classical description (e.g., foamy GCs).

Aim: To explore the morphological polymorphism of GCs in patients with GD type 1 (GD1).

Methods: May-Grünwald Giemsa stained bone marrow smears (BM-S), previously obtained from GD1 patients for diagnostic purposes, were analyzed in the ×40 objective of Olympus BX 40 microscope. Each patient sample consisted of 2 slides where all GCs and non-GC macrophages were counted. Features of GCs considered as atypical included: foamy cytoplasm (involving >10% of the cytoplasm), a centrally placed nucleus, GCs diameter >100 µm, multi-nuclear GCs, unusually large cytoplasmic projections, and apparent hemophagocytosis (Figure, b-h). Multi-nuclear GCs larger than 100 µm were classified as a syncytium. The diameter and radius of the cells were estimated by comparison with an erythrocyte (approx. 7 µm), a reticulocyte (7-10 µm) or a band/segmented granulocyte (approx. 14 µm). The non-GC macrophages were identified based on the absence of the fibrillary structure of the cytoplasm without being foamy, though they could contain a few vacuoles.

Results: A total of 12 BM-S from 6 patients (2 female, 4 male) with untreated GD1 were evaluated. The median age of the patients (pts) was 65 years (range 21–84 years), three of them (50%) were splenectomized. All but one patient (83%) carried at least one allele with a N370S (c.1226A>G) mutation in the *GBA1* gene. The median total number of GCs (typical and atypical) identified on 2 BM-S from one patient was 533 (range 132–2159 GCs) and the median percentage of atypical GCs among all GCs was 29% (range 22–40%). The median total number and percentage of non-GC macrophages was very low as compared to GCs (2, range 3–17 and 1%, range 1–2%, respectively). The repertoire of atypical features of GCs in the BM-S ranged from 6 to 13 per patient (median 10 atypical features). GCs with multinuclearity and GCs presenting with erythrophagocytosis, phagocytosis of erythroblasts, thrombocytes and unidentified nuclear cells, could be found in all patients. Foamy cytoplasm could be found in all but one patient (median value 6% of atypical GCs, range 0–15%). GCs with a centrally placed nucleus could be found in 5 (83%) pts (median value 1% of atypical GCs, range 0–2%); all of them had also foamy GCs. GCs with syncytial morphology were only found in 2 (33%) pts. Multinuclearity was found in all pts, the proportions ranged from 5–18% of atypical GCs (median of 12%). Cytoplasmic projections could be found in 5 pts (median value 0.5% of atypical GCs,

range 0–2%). Erythrophagocytosis could be found in all pts ranging from 5–15% of atypical GCs (median value 6%).

Discussion: The results suggest that untreated patients with GD1 often show a reasonable proportion of GCs with atypical cell morphology. Initial symptoms of Gaucher disease (e.g., thrombocytopenia, splenomegaly) among non-Jewish patients with sporadic GD1 often lead to hematological diagnostics, including bone marrow examination. Therefore, knowledge of possible atypical variant forms of GCs in BM-S can contribute to a quicker and accurate GD diagnosis, and minimize the risk for misdiagnosis (e.g., hemophagocytosis, other storage disease such as Niemann-Pick disease, Wolman disease, adult cholesteryl ester storage disease /CESD/, etc).

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Poster 16

Analysis of efficacy and safety of two iron chelator in type 1 Gaucher disease patients with iron overload.

Medrano B^{1,2}, Irun MP^{3,4}, Sarria L⁵, Alfonso P^{1,2,3}, Pocovi M^{2,3,4}, Giraldo P^{1,2,3,6}.

¹Traslational Research Unit. Miguel Servet University Hospital. Zaragoza. Spain. ²Aragon Health Sciences Institute (I+CS). Zaragoza. Spain. ³Biomedical Network Research Center on Rare Biology and Diseases (CIBERER), ISCIII. Zaragoza. Spain ⁴Department of Biochemistry and Cellular and Molecular Biology. University of Zaragoza. Spain ⁵Department of Radiology. Miguel Servet University Hospital. Zaragoza. Spain. ⁶Service of Hematology. Miguel Servet University Hospital. Zaragoza. Spain.

Serum ferritin was found to be elevated in type 1 Gaucher disease (GD1) in more than 60% of patients at diagnosis. Patients with GD1 have compromised the reticuloendothelial macrophages function that induce an inflammatory situation and as consequence a dysregulation in the iron storage and recycling. Excess iron injures cells primarily by catalyzing the production of reactive oxygen species in excess.

The aim of study is to quantify the iron overload in liver by MRI in GD1 patients with hyperferritinemia and its relation with the keys of disease.

Patients and Methods: 7 GD1 patients with severe iron overload (ferritin levels >500 ng/mL) were included in a clinical open randomized trial to evaluate the efficacy of two different iron quelators in subjects with iron overload during four months. The analysis included physical exam, blood counts, iron profile, protein profile, proBNP concentration, chitotriosidasa activity and CCL18/PARC, study of HFE genes, quantitation of iron liver deposits by MRI, calculating the ratio of strength signal between liver parenchyma vs the paravertebral muscles (LIC) and QOL.

Results: In 7 adults GD1 patients, mean age 49.8 y (29-75) 3 females and 4 males, only two patients were esplenectomized, 3 patients do not received any kind therapy related GD and 4 had received different therapies. Genotype: 1 N370S/N370S, 2 N370S/L444P, 1 N370S/RecNciI, 1 N370S/c.500insT, 1 N370S/V398I, 1 S13L/G202R. Related to HFE genes 1 patient was heterozygous to H63D mutation and other one to C282Y, the other 5 had not mutations. Mean hemoglobin: 13.4 g/dL (12.3-15.0), mean platelets: 100x10⁹/L (66-130), Mean ferritine at baseline: 941.85 ng/mL (677-1184), ProBNP 92.6 pg/ml (13.4-271) LIC: 24.2 (5-55). Chitotriosidase activity, CCL18/PARC. Four patients received Deferasirox 20mg/Kg/daily p.o. and 3 Deferoxamine 30 mg/kg sc three times/week. After 4 months on therapy mean ferritine 415.4(214-773), ProBNP 115.0 pg/ml (36.4-263), LIC:10(0-45).

Conclusions: No differences in severity iron overload between patients heterozygous for HFE genes or normal. Significant decrease of ferritine and iron liver deposits after chelation therapy. We have not observed differences in ProBNP concentration. The relation between ferritine decrease and plasma biomarkers of activated macrophage: Chitotriosidase, CCL18/PARC will be provided in the meeting. Acceptable tolerance to chelation therapy, without relevant adverse effects. Quality of life: Improvement in patients under oral therapy compared with parenteral iron chelation therapy.

Poster 17

Evaluation of norrbottnian Gaucher disease (type 3) patients after dose reduction of Imiglucerase therapy due to the worldwide supply shortage of Cerezyme® during 2009-2010.

Kämpe Björkqvall C¹, Wieremiejczyk J¹, Myhr-Eriksson K¹, Hägglund H², Machaczka M².

¹Department of Medicine, Sunderby Regional Hospital of Norrbotten County, Luleå, Sweden and ²Department of Medicine at Huddinge, Karolinska Institutet, Stockholm, Sweden.

Introduction: Although neuronopathic forms are the rarest variants of pan-ethnic Gaucher disease (GD), an endemic cohort of Swedish patients (pts) with chronic neuronopathic GD (GD3) lives in Northern Sweden in the county of Norrbotten, inhabited by 250,000 people on the area of approx. 100,000 square km. This unusual form of GD3 is called the Norrbottnian form of GD3 (N-GD3) and consists of approx. 40% of all known cases of GD in Sweden. Due to the worldwide supply shortage of Cerezyme® during 2009–2010, which was a consequence of viral contamination (vesivirus 2117) of the production plant in June 2009, N-GD3 pts received reduced doses of enzyme replacement therapy (ERT) with imiglucerase (Cerezyme®) during the 14 month period.

Aim: To retrospectively analyze the effects of the imiglucerase shortage in adults with N-GD3.

Patients and Methods: There were 15 pts with N-GD3 in the county of Norrbotten in June 2009. Of these, 12 pts were adults (6 F, 6 M), aged 21–58 years (median age 42 years), 11 pts were splenectomized (2 pts partially) at a median age of 7 years (range 1–20 years), 7 pts had epilepsy, and 2 pts underwent allogeneic stem cell transplantation and were free of ERT. A total of 10 pts (4 F and 6 M) have used imiglucerase in June 2009; the median duration time of ERT was 17 years (range 8–17 years). The patient files were reviewed for collection of relevant clinical data. Analyzed variables included plasma chitotriosidase activity (control range: <40 nkat/L), plasma concentration of chemokine (C-C motif) ligand 18 (CCL18) (control range: <100 µg/L), whole blood hemoglobin concentration (Hb), whole blood platelet count (PLT), patients' subjective complaints and body weight.

Results: A total of 9 adult patients were evaluated regarding the effects of an unintended dose reduction of imiglucerase; one patient died in Nov 2009 due to esophageal cancer and was not included in the analysis. The absolute dose reduction was from the median of 2800 IU (range 1600–4000 IU) to the median of 1650 IU (range 1200–2000 IU). The median proportion of ERT reduction was 63% (range 41-74%) compared with the imiglucerase dosage before June 2009; ERT dose reduction was uncertain in one patient due to his self-administration of imiglucerase. At the end of the shortage period chitotriosidase activity increased in 2/9 pts, concentration of CCL18 increased in 5/9 pts, Hb decreased in 5/9 pts, PLT decreased in 2/9 pts, and the body weight decreased in 1/9 pts. However, all above mentioned changes were minimal. Two pts were switched to velaglucerase (VPRIV®) after one year of imiglucerase shortage. One patient developed epilepsy at the age of 45 years, 22 months after the dose reduction of ERT.

Discussion: The results of our retrospective analysis suggest that the unintended dose reduction of imiglucerase lasting for a period of approx. 1 year has not lead to significant worsening in the GD blood biomarkers in adult patients with N-GD3. However, we cannot exclude that in one patient the reduced ERT was a contributing factor in the development of epilepsy (in an indirect mechanism).

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Poster 18

Ph-positive chronic myeloid leukemia in patient with Gaucher disease.

Sysoeva E, Chavinchak R, Turkina A, Lukina E, Domracheva E.

Hematology Research Center of Ministry of Healthcare and Social Development of the Russian Federation, Moscow, Russia.

Introduction: Gaucher patients having abnormal metabolism also can suffer from other diseases that should be treated without interrupting enzyme replacement therapy (ERT). We report such a case that was successfully treated.

Case Report: The female patient was born in 1946. Gaucher disease (GD) was diagnosed at the age of 27 years (in 1973). The patient did not receive ERT and had no complaints up to year 2003. Since 2003 the patient's condition deteriorated.

In December 2004 the patient was admitted to the Hematology Research Center in severe condition: weight 48 kg (height 158 cm), significant signs of anemia (60 g/l), hemorrhagies on skin, giant spleen occupying nearly the whole abdomen cavity. The diagnosis of GD was confirmed by enzyme assay. ERT with imiglucerase was started in December 2004. Several months later the patient's condition improved significantly, hemoglobin (Hb) and platelet levels increased.

Since May 2006 – deterioration: fatigue, enlargement of spleen, neutrophilic leukocytosis with the shift to the left. Bone marrow puncture and biopsy revealed myeloid metaplasia and Ph-chromosome found in 92% of the cells. Thus, Ph-positive chronic myeloid leukemia (CML) in the patient with GD was diagnosed. Treatment with imatinib was started. The patient was still receiving ERT (30 U/kg). Complete hematological remission of CML was observed after 6 months of treatment (Hb 120 g/l, Platelets $60,0 \times 10^9/L$, Leukocytes $5,0 \times 10^9/L$). However, there were still 3% of Ph-positive cells.

During the next 6 months the patient has not received treatment with imatinib (till November, 2007). She was feeling well and received 15-30 U/kg/infusion of ERT regularly. The spleen reduced significantly: +1-2 cm below costal margin. However, the control bone marrow puncture revealed 60% Ph+ cells, so the treatment with imatinib was started again (in December 2007).

At present time we observe hematological and molecular remission of CML (0,04% BCR-ABL cells), the patient has been receiving treatment with imatinib (400 mg daily) for 6 years. The goals of treatment of Gaucher disease are achieved, there is no spleno- and hepatomegaly, Hb 120g/L, Pl. $70 \times 10^9/L$, L. $4,0 \times 10^9/L$. The patient continues to receive the low dose of imiglucerase biweekly, is feeling well and leads normal active life.

Discussion: This case demonstrates huge possibilities of the modern anticancer treatment in combination with ERT in the patient with GD.

Poster 19

Ferritinemia during type 1 Gaucher disease: Mechanisms and progression under treatment.

Mekinian A, Stirnemann J, Belmatoug N, Heraoui D, Fantin B, Fain O, Charpentier A, Rose C.

Service de Médecine Interne, Centre de Référence des Maladies Lysosomales, Hôpital Jean-Verdier, AP-HP, Université Paris XIII, Avenue du 14 juillet, 93140 Bondy, France.

Background : Earlier results highlighted hyperferritinemia during type-1 Gaucher disease (GD), but its potential mechanisms and long-term progression remained unexamined.

Methods : We analyzed the clinical, biological and iron characteristics of type-1 GD patients, before and after starting enzyme-replacement therapy (ERT). Iron parameters under ERT were subjected to linear-regression analyses.

Results : Serum ferritin (median 739 [46-2371] $\mu\text{g/L}$) was determined for 54 patients (21 (39%) males; median age 32 [range 12-73] years) before ERT; it exceeded 300 $\mu\text{g/L}$ in 47 (87%), while the other iron parameters always remained normal: transferrin saturation coefficient (26 [16-42]), serum iron at 13 [6-22] mmol/L and transferrin at 2.4 [2,3] g/L . Four patients had mild elevation of liver transaminases, with C-reactive protein >20 mg/l in two. The absence of hemolysis was accompanied by a median bilirubin of 9 $\mu\text{mol/L}$ and lactate dehydrogenase at 250 IU/L ; diabetes and lipid anomalies were not observed. Clinical, biological and iron parameters at GD diagnosis were comparable for the 12 and 42 patients with ferritinemia ≤ 400 and $>400\mu\text{g/L}$, respectively. Ferritinemia was measured at least once for 46 patients after ERT onset (median treatment duration 90 [3-204] months). At study closure, median serum ferritin was 187.5 [11-1560] $\mu\text{g/L}$, exceeding 300 $\mu\text{g/L}$ in 15 (33%) patients, while the other iron parameters were normal. Among the latter, only the mean \pm SD ferritinemia slope decreased significantly under ERT (-1.9 \pm 0.3%/month; $p < 0.001$).

Conclusion : Hyperferritinemia is a specific GD characteristic and serum ferritin monitoring could be informative during follow-up.

Poster 20

Bone mineral density in adults with type 1 Gaucher disease receiving Velaglucerase alfa 60 U/Kg every other week : 2 year results.

Mehta A^a, Ben Turkia H^b, Kabra M^c, Lukina EA^d, Giraldo P^e, Kisinovsky I^f, Bavdekar A^g, Ben Dridi MF^b, Gupta N^c, Kishnani PS^h, Elstein Dⁱ, Zahrieh D^j, Hangartner TN^k, Crombez E^j, Zimran Aⁱ.

^aRoyal Free Hospital, London, UK; ^bLa Rabta Hospital, Tunis, Tunisia; ^cAll India Institute of Medical Sciences, New Delhi, India; ^dHematology Research Center, Moscow, Russia; ^eCIBERER and Hospital Universitario Miguel Servet, Zaragoza, Spain; ^fYour Health SA, Buenos Aires, Argentina; ^gKEM Hospital Research Centre, Pune, India; ^hDuke University Medical Center, Durham, NC, USA; ⁱShaare Zedek Medical Center and Hebrew University-Hadassah Medical School, Jerusalem, Israel; ^jShire Human Genetic Therapies, Inc., Lexington, MA, USA; ^kWright State University, Dayton, OH, USA

Introduction : As bone pathology is a significant complication in GD1, the effects of enzyme replacement therapy (ERT) with velaglucerase alfa on bone mineral density (BMD) were investigated over a 2-year treatment period.

Aim : To assess BMD in adult GD1 patients over 2 years of treatment with velaglucerase alfa at 60 U/kg every other week (EOW).

Methods : Dual-energy x-ray absorptiometry of the lumbar spine (LS) and femoral neck (FN) was used to assess BMD in adults in 2 pivotal Phase III trials (TKT032, HGT-GCB-039) and an extension study (HGT-GCB-044). BMD Z- (age and sex matched) and T-scores were calculated.

Results : 19 adult GD1 patients (median age: 31 [range: 19–58 years]; 11 females, 8 males) completed 2 years of ERT with velaglucerase alfa and no concomitant bisphosphonates. Median (range) LS and FN Z-scores at Baseline (BL) were -1.59 (-4.20–0.98) and -1.2 (-3.53–2.84), respectively. The mean change (95% CI) in Z-score from BL at 1 year was statistically significant for LS (P<0.05): 0.27 (0.10, 0.44), but not for FN: -0.02 (-0.25, 0.21). The mean change (95% CI) in Z-score from BL at 2 years was also statistically significant for LS (P<0.05): 0.64 (0.35, 0.93), but not for FN: 0.1 (-0.12, 0.32). When an additional 2 patients (18 and 60 years of age; 1 male, 1 female) who received concomitant bisphosphonates were included, the mean change (95% CI) in the LS Z-score from BL at 1 and 2 years remained statistically significant (P<0.05) at 0.23 (0.06, 0.40) and 0.58 (0.31, 0.86), respectively, whereas the mean change (95% CI) in the FN Z-score from BL at 1 and 2 years was non-significant (-0.01 [-0.23, 0.20] and 0.16 [-0.07, 0.40], respectively). According to the WHO classification based on LS T-scores at BL, 4/19 patients had normal bone density, 10 were osteopenic and 5 were osteoporotic. By 2 years, 5/19 patients changed LS WHO classification from osteopenia to normal and 1 patient changed from osteoporosis to osteopenia. For the WHO classification based on BL FN T-scores, 10/19 patients were classified as normal, 8 were osteopenic and 1 was osteoporotic; by 2 years, 1/19 patients changed classification from osteopenia to normal, another changed from osteoporosis to osteopenia, 2 patients changed from osteopenia to osteoporosis, and 1 changed from normal to osteopenia.

Discussion : Beginning at 1 year, a statistically significant and clinically meaningful improvement in LS BMD was achieved among adult GD1 patients treated with velaglucerase alfa 60 U/kg EOW.

Poster 21

Evaluation of bone marrow involvement using a semi-quantitative magnetic resonance imaging method in patients with non-neuropathic Gaucher disease.

Komninaka V¹, Kolomodi D², Mitropoulou M², Marinakis T³, Repa K⁴, Michelakakis H⁵, Voskaridou E¹, Christoulas D⁶, Terpos E⁶.

¹Department of Thalassemia, Laikon General Hospital, Athens, Greece; ²Department of Radiology, St Savvas Oncology Hospital, Athens, Greece; ³Department of Hematology, "Georgios Gennimatas" General Hospital, Athens, Greece; ⁴Department of Hematology, Polykliniki General Hospital, Athens, Greece; ⁵Department of Enzymology and Cellular Function, Institute of Child Health, Athens, Greece; ⁶Department of Clinical Therapeutics, University of Athens School of Medicine, Athens, Greece.

Introduction: Non-neuronopathic form of Gaucher disease (GD) is accompanied by bony complications including marrow infiltration and lytic lesions. MRI is the most sensitive imaging procedure for assessing skeletal involvement in GD patients.

Aims: To develop a semi-quantitative scoring system, based on MRI, for the evaluation of the bone marrow involvement in non-neuronopathic GD.

Methods: We studied 27 adult patients (15M/12F, median age 44.5 years) with non-neuronopathic GD and 27 healthy controls. MRI of the femoral bones was performed in all patients and controls. We implemented T1-weighted spin echo (T1W) sequences, proton density sequences with fat suppression (PDFS) to further evaluate the detected changes on T1W sequence and short time inversion recovery (STIR) sequences for the evaluation of disease activity. We also performed a thorough comparison of T1W and PDFS images targeting in the detection of the remodeling process of the affected bone. In all patients we evaluated bone infiltration on T1W images in the affected areas as opposed with those of healthy regions and in conjunction with measurements taken from subcutaneous fat of both thighs of the same patients.

Results: The MRI findings were independently evaluated by two experienced MRI specialists. The rate of subcutaneous fat of both healthy and affected individuals was fluctuated from 210 to 400 pixels and those of bony fat tissue from 120 to 180 pixels. On the contrary the affected values ranged from 15 to 80 pixels. In order to reduce the possibility of false positive and false negative values we combined all the region of interest (ROI) measurements with the degree of infiltration detected on T1W images. At the end we combined the above MRI findings and introduced the following classification: stage I: ROI 1/2 of normal values and bone infiltration up to 30%; stage II: ROI 1/3 of normal values and bone infiltration from 30 to 60%; stage III: ROI 1/4 of normal values and bone infiltration from 60% to 80% and stage IV: detection of epiphyseal infiltration, osteonecrosis and deformity regardless of the ROI'S values. All but one patient had abnormal MRI findings: 3 (11%) had stage I MRI abnormalities, 18 (66%) had stage II, 3 (11%) stage III and 2 (7%) stage IV.

Discussion: The above semi-quantitative MRI method is relatively easy to be performed compared to other available methods for the non-neuronopathic GD and would be useful for the evaluation of bone marrow involvement in this GD type.

Poster 22

Unusual bony complications in enzyme replacement treated type 1 Gaucher patients.

Rosenbaum H.

Department of Hématologie & Bône Marrow Transplantation ; Rambam Health Care Campus, Bruce Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, Haifa Israel.

Introduction : Bone involvement is common in Type 1 Gaucher disease(GD) usually include Gaucher crisis, bone pain, osteoporosis, osteosclerosis, lytic lesions, pathological fractures, avascular necrosis(AVN) and asymptomatic bone remodeling abnormality causing the typical Erlenmeyer flask deformity of the long bones.

Enzyme replacement therapy (ERT) prevents bone crisis, alleviates pain and has also a beneficial effect on bone marrow infiltration by Gaucher cells. ERT has only minor effect on existing AVN, osteolytic lesions and pathological fractures.

Aim : To report cases of unusual bony manifestations among GD patients treated with ERT for almost twenty years.

Methods : The clinical data of 44 GD ERT treated patients followed at the Gaucher clinic in the Rambam Health Care Center in Haifa, Israel were evaluated for atypical bone complications.

Results : In 3/44 GD patients treated by ERT since 1991 unusual bony complications were observed. Two of the patients presented more than one type of atypical bone involvement. Patient 1 presents multiple asymptomatic osteolytic lesions in both mandible bones with no other bone symptoms or evidence of osteopenia and AVN.

Patient 2 suffers from painful lytic lesions and pathological fractures in navicular, capitatum and lunate bones as well as a Gaucheroma in the Tibia.

Patient 3 suffers from mandibular lytic lesions and multiple bilateral pathological fractures in the ribs. Interestingly, all three patients present atypical bony complications following over 15 years of ERT, while the visceral and hematological phenomena resolved years before. None of the three patients underwent splenectomy prior to ERT.

Conclusion : The occurrence of unusual bone features in long term ERT treated GD patients should be further investigated using specific bone evaluation tests and biomarkers.

Poster 23

Hyperuricemia in type 1 Gaucher disease: Is uric acid a biomarker for disease severity?

Watad S, Rosenbaum H.

Department of Hematology & Bone Marrow Transplantation ; Rambam Health Care Campus, Bruce Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, Haifa Israel.

Introduction : Elevated levels of Uric Acid (UA) were observed in Type 1 Gaucher Disease (GD) patients followed at the Gaucher Clinic in Rambam Medical Center. To our knowledge there are no previous reports regarding the frequency of hyperuricemia in Type 1 GD patients.

Aims : To estimate the frequency of hyperuricemia in Type 1 GD patients, and correlate the levels of uric acid to disease severity and concurrent lymphoproliferative disorders (LPD) and other malignancies.

Methods : A cohort of 69 Type 1 GD patients, 25 naïve patients and 44 on Enzyme Replacement Therapy (ERT), followed at the Gaucher Clinic in the Hematology Department in the Rambam medical center in Haifa, Israel was evaluated for UA levels, kidney function tests, biomarkers and the presence of concomitant lymphoproliferative disorders or other malignancies. Disease severity score was evaluated according to Zimran's severity index (SSI) and related to levels of UA.

Results : The study cohort comprises 69 patients with Type 1 GD, 36(52%) females and 33(48%) males, followed for up to 20 years at our center. The mean age of patients was 51.59 ± 16.19 years (range 21-87 years). Mean severity score index was 8.94 (range 1-21). Levels of UA were significantly higher in patients with a severe disease course (SSI 11.6 ± 4) compared to patients with mild disease (SSI 4.48 ± 2.84), 5.83 ± 1.6 mg/dl and 4.89 ± 1.43 mg/dl respectively, $p=0.032$. A linear and statistical significant correlation was found between UA levels and disease severity (according Zimran's Severity Score Index), $p=0.042$. A linear correlation was also detected between levels of Acid Phosphatase (AP) and UA $p=0.038$. No impairment in renal function tests were observed in patients with high UA. There was no statistically significant difference between uric acid levels observed in GD patients with LPD and GD patients with no LPD. An increase in UA was observed prior to and during ERT in patients with severe GD 5.83 ± 1.6 and 6.56 ± 2.33 , respectively, $p=0.054$. Interestingly, the effect of ERT was more pronounced in the male cohort, with UA $=6.472 \pm 1.55$ mg/dl before ERT, compared to 7.483 ± 2.16 mg/dl, during therapy, $p=0.011$.

Conclusions : High UA level may be used as a marker of Gaucher cell burden and Type 1 GD severity. Uric acid should not be used as a predictive factor for concomitant LPD and malignancies in patients with GD. The elevated UA values during ERT might relate to increased Macrophages (Gaucher cells) turn over.

Poster 24

Inherited thrombophilia in Gaucher patients with avascular necrosis of the Hip.

Lukina K, Sysoeva E, Mamonov V, Yatsyk G, Lukina E, Sudarikov A.

Hematology Research Center of Ministry of Healthcare and Social Development of the Russian Federation Moscow, Russia.

Introduction: Intravascular coagulation appears to constitute a pathogenetic mechanism through which various environmental and genetic risk factors lead to bone ischemia and necrosis. Recent studies have suggested that genetic polymorphisms in factor V, prothrombin and methylenetetrahydrofolate reductase (MTHFR) genes leading to intravascular coagulation disorders may be related to avascular necroses of the hip in Gaucher patients. Inherited thrombophilia is diagnosed when the homozygous mutation of MTHFR and/or heterozygous mutation of factor V and/or prothrombin are found. We hypothesized that inherited thrombophilia might be a risk factor for avascular necrosis of the hip in Gaucher patients.

Aim: To study genetic polymorphisms in factor V, prothrombin and MTHFR genes in patients with Gaucher disease type 1 and evaluate the correlation between the presence of avascular necrosis of the hip and markers of inherited thrombophilia in Gaucher patients.

Materials and Methods: The group included 152 adult (55 men, 97 women) Gaucher patients. The diagnosis was verified by enzyme assay in all patients.

PCR analysis was used to study three heritable thrombophilic gene mutations: heterohomozygosity for the G1691A Factor V Leiden, G20210A prothrombin gene and homozygosity for the C677T MTHFR mutation.

The diagnosis of avascular necrosis of the hip was based on clinical data and was documented by radiographs of both hips and MRI evaluation.

Results: Mutations in MTHFR gene were found in 69 (45%) pts, 10 (9%) pts had homozygous mutations. Mutations in prothrombin gene were detected in 4 (2,6%) pts, in factor V gene – in 7 (4,6%) pts. Five pts had a combination of 2 mutations. A combination of 3 heterozygous mutations was found in 1 patient.

Avascular necrosis of the hip was diagnosed in 32 (21%) pts.

Only 2 (6%) patients with avascular necrosis of the hip were diagnosed with inherited thrombophilia. The prevalence of inherited thrombophilia in the group of Gaucher patients without avascular necroses was 14% (17 patients).

Conclusions: No correlation was found between the presence of avascular necrosis and markers of inherited thrombophilia in our cohort of Gaucher patients.

Poster 25

Blood circulating monocytes in Gaucher disease : Are they effective pharmacological targets for recombinant enzymes?

Berger J¹, Bourgne C¹, Stirnemann J², Pereira B³, Pigeon P¹, Heraoui D⁴, Rapatel C¹, Rose C⁵, Belmatoug N⁴, Berger MG¹.

¹Hématologie Biologique, CHU Estaing, 1 place Lucie Aubrac, 63003 Clermont-Ferrand Cedex 1, France.

² Médecine Interne, CHU Jean Verdier, AP-HP, Avenue du 14 juillet, 93143 Bondy, France

³ Direction de la Recherche Clinique et des Innovations (DRCI), CHU G Montpied, 58 rue Montalembert, 63003 Clermont-Ferrand Cedex 1, France

⁴ Médecine Interne, Hôpital Beaujon, AP-HP, 100 Bd Général Leclerc, 92110 Clichy, France

⁵Onco-Hématologie, Hôpital Saint-Vincent de Paul, Boulevard de Belfort, 59000 Lille, France

Introduction : Two new biosimilar agents, velaglucerase-alfa (VEL) and taliglucerase-alfa (TAL) have been described as being similar to Imiglucerase (IMI), the first line treatment of type 1 GD patients, but differing slightly in glycan structure which could have an impact on macrophage uptake and thus on therapeutic efficacy. However, the ability of native GD blood monocytes (Mo) to capture recombinant enzymes (REs) remains unknown.

Patients and methods : The left-over part of biological samples collected for routine analysis from type 1 Gaucher patients (n=4) were used for research because patients had been informed and did not express any disagreement. Mononuclear cells were incubated with three concentrations of each RE (0.1, 0.5 and 1 U/ml) at 37°C for 30 min and one hour and washed twice with PBS before evaluation of intra-Mo (IMo) GC activity (GDA) by standardized flow cytometry.

Results : We observed a dose-dependent *in vitro* uptake of IMI, TAL and VEL in Mo from 4 untreated GD patients, but uptake of TAL was systematically lower than that of IMI and VEL.

Case analysis showed an inter-patient heterogeneity, with the highest increase of IMo GCA for all REs in patient #2 and the lowest in patient #3; this observation was confirmed *in vivo* by analysis of Mo 15 min after the end of the first IMI infusion (x 38 and x 9 endogenous IMoGCA respectively) whereas patient #2 had received a lower dose of enzyme. Patient #2 improved thrombocytopenia (68%) and increased hemoglobin level (+1.9g/dL) from M3, but hematological response for patient #3 could not be compared because she had been splenectomized 20 years ago. Because of the chitotriosidase deficiency of patient #2, we used plasma CCL18 as a biomarker; decrease kinetics of CCL18 was more rapid for patient #2. Similarly, correction of glycosylated-ferritin was better in patient #2. The two other patients with IMoGCA values close to that of patient #2 did not require treatment (patient #1), or had thrombocytopenia that improved over the expected period of time (patient #4).

Discussion : In conclusion, this study shows inter-patient variability in the ability of blood Mo to store recombinant enzymes that was confirmed *in vivo*. Interestingly, patient #2 had a mild form of GD, suffering only from thrombocytopenia and asthenia, while patient #3 had an aggressive form of GD, with bone disorders. We hypothesize that this variability could partially explain the heterogeneity of GD response to enzyme replacement therapy.

Poster 26

Monocytes in Gaucher's disease patients: Quantitative and functional impairments.

Bettman N¹, Avivi I^{1,2}, Katz T², Rosenbaum H^{1,2}.

¹Bruce Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, Haifa, Israel ²Department of Hematology & Bone Marrow Transplantation, Rambam Health Care Campus, Haifa, Israel

Introduction: Gaucher's Disease (GD) is characterized by glucocerebroside (GC) accumulation due to defective activity of the glucocerebrosidase (GBA) enzyme. Amongst hematopoietic cells, monocytes (Mo) and macrophages exhibit the highest GBA activity. Monocyte recruitment and differentiation play a critical role in defense mechanisms against pathogens and in inflammatory response. GC accumulation may impair their function.

Aims: To investigate monocytes' function in GD patients.

Methods: 24 untreated GD patients and 14 healthy volunteers (HV) were recruited to the study (IRB number: 3027). Peripheral blood mononuclear cells (PBMCs) were analyzed for their monocytes' content and migration capabilities. In the migration test, PBMCs were allowed to migrate through 5µm inserts for 4 hours towards medium containing stromal cell-derived factor 1 (SDF1), and the percentage of migrating Mo was determined by FACS. Following isolation of CD14⁺ cells, the expression of the SDF1 receptor, CXCR4, was evaluated by FACS and its mRNA was determined by qPCR. Serum SDF1 concentrations were quantified by ELISA.

Results: The number (cells/ml) of Mo in peripheral blood (PB) was significantly lower in GD patients (n=14) compared with HV (n=13; 5.3×10^5 vs. 9×10^5 ; $p=0.018$). In addition, GD-derived Mo (GD-Mo; n=9) showed a significant reduced migration capacity in response to SDF1, compared with that obtained for HV-derived Mo (HV-Mo; n=7; 6.3% vs. 14% and 9% vs. 21.8% for 500 & 1000 ng/ml SDF1 concentrations, respectively; $p<0.05$). Staining of CXCR4 revealed that GD-Mo (n=11) express less CXCR4 than HV-Mo (n=7; 44.5% vs. 59.7%; $p<0.01$). However, qPCR analysis demonstrated that the amount of CXCR4 mRNA transcripts in GD-Mo (n=9) is similar to that obtained from HV-Mo (n=7; relative expression of 0.03 for GD patients vs. 0.034 for HV). Serum SDF1 concentrations were elevated in GD subjects (n=10) compared with their healthy counterparts (n=10; 2603 pg/ml vs. 2039 pg/ml; $p<0.01$).

Discussion: GD patients' exhibit quantitative and qualitative impairments, demonstrated by their decreased migration capacity towards SDF1. The impaired migration capacity may be at least partly related to the decreased expression of CXCR4 on patients' Mo, induced in response to the high SDF1 serum levels found in these patients. The impaired migratory capacity observed, may contribute to the abnormal immune function reported in GD patients, though this issue should be further verified and explored.

Supported by the Gaucher Generation Grant, Genzyme Corporation.

Poster 27

Potential biological advantage of Gaucher disease: antimicrobial properties of the accumulating lysolipids.

Roos JCP, Cox TM.

Department of Medicine, University of Cambridge.

Introduction : A selective advantage for Gaucher disease heterozygotes and homozygotes has been proposed based on the high gene frequency of this disease. However, there has been no evidence forthcoming as to what this selective advantage might be. To date, the role of resistance to infection, has been proposed but no evidence of any advantage discovered.

Aim : To explore microbial susceptibility to low concentrations of bioactive lysosphingolipid Gaucher metabolites.

Methods : Gram positive and negative bacilli and cocci were incubated with sub-pathological concentrations of sphingolipids not shown to affect cell morphology.

Results : β -glucosylceramide and ethanol vehicle control have no effect on culture density, 2.5 μ M psychosine essentially sterilized cultures of *Listeria monocytogenes*. Though this may at first appear a large concentration, Gaucher spleen is known to contain up to 160 μ M glucosylsphingosine assuming uniform distribution. Experiments showed that adherent cells in culture can tolerate tenfold greater concentrations without any phenotypic effect. Resuspension in fresh media allowed colonies to re-expand suggesting that psychosines are bacteriostatic rather than bacteriocidal. The effect on other types of bacteria was assessed as a broad resistance to microbial infection would greatly increase selection for Gaucher's. *Escherichia coli* was also inhibited by psychosine, but was more resistant than *Listeria* and might not be selectively disadvantaged compared to human cells. Experiments with a third bacterium, the gram negative anaerobic bacillus *Bacteroides fragilis* showed that it appears sensitive to psychosine but as it was not cultured in an anaerobic environment this may have rendered it less able to deal with additional adverse conditions.

Discussion : These experiments suggest a potential selective advantage for patients with some glycosphingolipid disorders. Modest accumulation of a cationic amphiphile may provide an advantage for patients with Gaucher disease by reducing the survival of *Listeria*. Fungi generate highly active ionophores with antimicrobial properties, such as ionomycin from *Streptomyces* but here we provide evidence for antimicrobial properties of Gaucher-related sphingolipids; it remains unclear as to whether heterozygous carriers would benefit from these properties.

Poster 28

The role of enzymology in determining Gaucher status in Parkinson's disease.

Burke D, Vellodi A, Heales S.

Clinical & Molecular Genetics Unit, UCL Institute of Child Health. Enzyme & Metabolic Unit, Great Ormond Street Hospital, London WC1N 3JH, UK

Since 1996 it has been recognised that there is an association between PD and GD [1]. The odds ratio for any GBA1 mutation in PD patients versus controls is 5.43[2]. Furthermore GD heterozygote status is now also known to be the strongest genetic risk factor for PD (4-5% of sporadic PD). As a result it is becoming routine to screen patients with Parkinsonism for GD using mutation analysis and/or enzyme activity.

Methods : The b-glucosidase (GBA1) activity of samples from 69 adult patients being investigated for dystonia or Parkinsonism was analysed. Only samples with a normal b-galactosidase level (control enzyme) were included.

A pooled normal leucocyte pellet was also incubated with 3-methyldopa (3MD) prior to b-glucosidase estimation to ascertain whether this principal L-DOPA metabolite could affect b-glucosidase activity in PD patients receiving L-DOPA.

Results : 29 % of this highly selective population were in the heterozygote range of enzyme activity. 62% were in the overlap range between heterozygote and unaffected status so may or may not be heterozygotes. Two of these patients had the E326 mutation. There was no significant difference in the age of the males and females or their GBA 1 activity. There was no correlation between GBA 1 activity and age or GBA1: BGAL ratio and age.

Discussion : Enzyme activity in the heterozygote range can be used to identify patients for mutation analysis. However patients in the heterozygote/normal overlap range should not be excluded from mutation analysis. Whilst enzyme activity may indicate heterozygote status it is recognised that some PD patients may have lower levels of enzyme due to the disease process [3, 4].

L-DOPA therapy is unlikely to have an effect on enzyme activity as no effect was observed with up to 2 mm 3MD *in vitro*.

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Poster 29

Efficiency and safety of velaglucerase in patients with Gaucher's disease : the French experience.

Serratrice C¹, Bengherbia M², Kaminsky P³, Berger M⁴, Grosbois B⁵, Leguy-Seguin V⁶, Belmatoug N².

On behalf of the CETG⁷ : Billette de Villemeur T, Broissand C, Camou F, Caillaud C, Froissart R, Heraoui D, Kettaneh A, Levade T, Masseur A, Mignot C, Robert A, Rose C, Sedel F, Stirnemann J, Valyannopoulos V, Vanier MT.

(1) Médecine Interne, Fondation Hôpital Saint Joseph, Marseille, France ; (2) Service de Médecine Interne, Hôpital Beaujon, Clichy, France ; (3) Service de Médecine J, Hôpitaux de Brabois, Vandoeuvre les Nancy, France ; (4) Laboratoire Hématologie CHU Clermont Ferrand, (5) Service de Médecine Interne, CHU Rennes; (6) Service de Médecine Interne, CHU Dijon ; (7) Comité d'Evaluation du Traitement de la maladie de Gaucher.

Introduction : Imiglucerase, is currently used since 20 years in Gaucher's disease (GD). Supplying difficulties for imiglucerase occurred during 2009, new enzymotherapies, including Velaglucerase, were available. The aim of our study is to report clinical and biological evolution during velaglucerase treatment in France

Patients and methods : This retrospective study includes all french patients treated with velaglucerase. Velaglucerase was introduced after switch or at baseline. Hemoglobin, platelets count, chitotriosidase and ferritin were monitored before treatment, at the initiation of velaglucerase and during the treatment. Clinical events were reported.

Results : Sixty patients are or have been treated with velaglucerase, 23 females and 37 males. Ten patients stopped the treatment. Nineteen patients were splenectomized. Eight patients had never been treated before. Others were treated by imiglucerase and for some by miglustat. Haemoglobin increased of 3%, platelets of 10%, chitotriosidase decreased by 47% and ferritin by 48%. Velaglucerase has been well tolerated.

Discussion : We observe even in patients stabilized by imiglucerase an increase of haematological parameters, and mainly a dramatical decrease of biomarkers.

Conclusion : Velaglucerase appears to be a safe and efficient alternative therapy to imiglucerase in GD patients.

Poster 30

Comparative analysis of Taliglucerase alfa efficacy with enzyme replacement therapies for Gaucher disease.

Chertkoff R¹, Paz A¹, Brill-Almon E¹, Fletcher N², Silverstone J³, Golembo M¹.

¹Protalix Biotherapeutics, Carmiel, Israel; ²Pfizer, Sydney, NSW, Australia; ³Pfizer, New York, NY, USA.

Introduction: Enzyme replacement therapy (ERT) for Gaucher disease (GD) can be assessed using various methodologies; one methodology incorporates a 3-category therapeutic response classification system. Taliglucerase alfa is a plant-cell-expressed United States Food and Drug Administration–approved ERT for GD.

Aim: This study utilized a 3-category classification system of good, moderate, or no response to compare the therapeutic responses of taliglucerase alfa with imiglucerase and velaglucerase alfa.

Methods: Responses were categorized with respect to changes from baseline of the following: Spleen or liver volume reduction—good ($\geq 30\%$), moderate ($\geq 10\%$ and $< 30\%$), no response ($< 10\%$); hemoglobin increase—good (≥ 1.5 g/dL), moderate (> 0.5 and < 1.5 g/dL), no response (≤ 0.5 g/dL); platelet count increase—good ($\geq 30 \times 10^9/L$), moderate ($> 15 \times 10^9/L$ and $< 30 \times 10^9/L$), no response ($\leq 15 \times 10^9/L$). The taliglucerase alfa analysis utilized data from pivotal trials PB-06-001 (patients randomized to 30 or 60 U/kg) and extension study PB-06-003 (continuation on same dose). Comparison of response rates was made for 60 U/kg at 9 months using data for velaglucerase alfa (HGT-GCB-039 and TKT-032) and imiglucerase (HGT-GCB-039) and at 12 months for velaglucerase alfa (TKT-032).

Results: At all time points and both doses of taliglucerase alfa, 100% of patients achieved a moderate or good response in spleen volume. At 24 months and 60/30 U/kg, respectively, the following response rates were observed: 100%/92% achieved a good spleen volume response, 100% of patients achieved a moderate or good response in liver volume (50%/22% achieved a good response), 88%/50% achieved a good hemoglobin response, 71%/42% achieved a good platelet count response. Analysis of response rates to taliglucerase alfa, velaglucerase alfa, and imiglucerase across the 4 clinical parameters were comparable at the common evaluation time point of 9 months; assessment of taliglucerase alfa and velaglucerase alfa at 12 months also revealed comparable response rates.

Discussion: In this retrospective analysis using a 3-category classification system, therapeutic response rates appeared to be comparable for taliglucerase alfa, imiglucerase, and velaglucerase alfa.

Poster 31

Innate and adaptativ dysfunction in Gaucher's type 1 disease are reverse by treatment.

Braudeau C^{1,2*}, Graveleau J^{3*}, Rimbert M^{1,2}, Hamidou M³, Grosbois B⁴, Neel A³, Josien R^{1,2*}, Masseau A^{3*}.

¹ CHU Nantes, Laboratoire d'Immunologie,

² INSERM, UMR1064, Nantes, France,

³ CHU Nantes, Service de Médecine Interne

⁴ CHU Rennes, Service de Médecine Interne, Rennes, France.

* *Authors contributed equally to this work.*

Background: Gaucher disease is caused by an autosomal-recessive deficiency of β glucocerebrosidase leading to an accumulation of glucosylceramide in monocytes/macrophage lineage. We analyzed immune cells and especially the function of dendritic cells to evaluate the potential impact of glucosylceramide accumulation in these cells and its possible role in infections and malignancies usually described in this pathology. The study was performed before and under replacement therapy.

Methods: Ten Gaucher patients were studied and compared with healthy volunteers. Immune cells (B cells, T cells, NK, dendritic cells), were studied by flow cytometry directly on whole blood. The innate function of dendritic cells, such as cytokine production, was assessed after stimulation by Toll-like receptors ligand. Cytokines in sera were measured using a multiplex assay.

Results: Gaucher patients displayed decreased numbers of NK cells, gd2 T cells and increased frequency of memory $CD4^+CD45RO^+$ T cells, when compared to healthy controls. Numbers of dendritic cells (mDC and pDC) were also decreased. We also demonstrated that pDC in GD exhibited a decrease IFN production after TLR9 stimulation compared to controls. Importantly, enzyme replacement therapy restored pDC function. Finally, we observed an increase of IL-8 and IL-18 in GD patient sera which was no longer observed under enzyme treatment.

Conclusions: Our data show that patients with GD exhibit altered number of innate and T lymphocytes as well as reduced DC numbers and altered pDC innate function. These modifications could participate to a decreased response to pathogens and favour malignancies.

Poster 32

MR imaging manifestations of skeletal involvement and effect of enzyme replacement therapy in adult patients with type I Gaucher disease.

Anastasiou A¹, Vasimidou K¹, Barmpas N¹, Liatopoulos G¹, Kyriakou D¹, Tsismalidou S¹, Papadopoulos P¹, Vakalopoulou S², Garypidou V².

¹ Department of Radiology, «Hippokratio» General Hospital, Thessaloniki, Greece,

² Department of 2nd Internal Medicine Clinic, Aristotle University, Thessaloniki, Greece.

Introduction/Aim: Gaucher disease (GD) is an autosomal recessive lysosomal storage disease in which glucocerebroside accumulates in cells of the reticuloendothelial system, due to a specific enzyme deficiency (glucocerebrosidase). Three subtypes exist, of which type I the non-neuronopathic, is the most frequent. The objective of our study is to investigate the morphology and detailed assessment of skeletal involvement and bone marrow changes in patients with GD type I and its response to the Enzyme Replacement Therapy (ERT).

Methods: Between 2002 and 2012, 7 adult untreated patients with type I GD (6 men, 17-73 years; 1 woman 42 years) who were referred to undergo evaluation of axial and peripheral skeleton for ERT and their follow-up were included in this study. A baseline MRI examination was performed before induction of ERT and at yearly intervals during ERT. The total follow-up period ranged from 9 months to 10 years. All patients had MRI examination on a 1.5T magnet (Symphony, Siemens Erlangen, Germany). All patients were investigated with sagittal spinal images and coronal images of pelvis and both femurs and tibiae with T1/T2-STIR weighted images.

Results: From the known semiquantitative staging and classification systems we classified our patients according to the system developed at the Institute of Diagnostic Radiology, Düsseldorf for the lower extremities and vertebra-disc ratio for the lumbar spine. All patients showed low signal intensity on T1/T2 images of the lumbar spine. One patient showed homogeneous (type A) and three heterogeneous (type B) loss of bone marrow signal of pelvis and lower extremities. All of them indicated a marrow response and improvement following ERT. Three patients showed homogeneous loss of bone marrow signal (type A), bone infarcts (type B) and infiltration of epiphyses, whereas one of them had 2 pathologic fractures of the lumbar spine and right femur. They indicated a negligible improvement during follow-up because they did not receive ERT despite our recommendation.

Discussion: When bone marrow is infiltrated by Gaucher cells, they cause reduction of signal intensity on both T1 and T2-weighted images homogeneously or heterogeneously in both axial and appendicular skeleton. The epiphyses are spared, becoming infiltrated only in cases of severe disease as in 3 of our patients. The heterogeneous pattern (type B) represents a higher incidence of irreversible bone changes as in our 3 patients with bone infarcts.

Poster 33

Bone disease, genetic and cytokines as markers in Gaucher disease.

Gervas-Arruga J^{1,2,7}, Roca M⁵, de Blas I⁶, Giraldo P^{1,2,3,7}, Pocovi M^{1,4,7}.

¹Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), ²Translational Research Unit. Miguel Servet University Hospital, ³Hematology Department. Miguel Servet University Hospital, ⁴Biochemistry and Molecular and Cellular Biology Department. Universidad de Zaragoza, ⁵Radiology (Magnetic Resonance) Instituto Aragonés de Ciencias de la Salud (I+CS), ⁶Unidad de Patología Infecciosa y Epidemiología, Facultad de Veterinaria, Universidad de Zaragoza. ⁷IISA. Zaragoza. Spain.

Introduction: Bone disease (BD) is one of the most debilitating and disabling complication of Gaucher disease (GD), leading to bone marrow infiltration by Gaucher cells, failure of remodelling, skeletal changes including osteolysis, osteopenia and intraosseous vascular complications. The most important factor involved in bone remodeling is the genetic component, which may explain between 60% and 80% of bone mass variability. In addition, Gaucher cells activate and induce proinflammatory cytokines synthesis that can modify the activity of osteoblast-osteoclast system and promote lytic phenomena.

Aim: To analyze the genetic variability in genes related to bone remodeling and to identify the cytokine profile in GD1 patients associated with BD in order to characterize bone markers in GD that can predict the degree of BD.

Methods: A total of 13 polymorphisms located in 5 genes (*COL1A*: -1997, *ESR1*: *PvuII*, *VDR*: *BsmI*, *ApaI*, *TaqI*, -1012, -1521, *BMP4*: 6007, *CICN7*: V418M, *IL6R*: -208, D358A, *OPG*: 950, 1181) were analyzed by RFLP. Furthermore a panel of cytokines: IL4, IL6, IL7, IL10, IL13, MIP1 α , MIP1 β and TNF α were analyzed in plasma samples by Luminex®100 platform and Millipore cytokine kits. A group of 34 GD1 patients with different BD degree were evaluated by MRI, calcaneus ultrasound and by calculating Bone Ultrasonic Attenuation (BUA): 18 without BD (females 38%, mean age: 40 year; range: 20-67), 16 with bone affection (females 56%; mean age: 46 years; range 14-75). The data were analyzed using Kolmogorov-Smirnov analysis, t-test or Mann-Whitney-U-test, one-Way ANOVA or Kruskal-Wallis test and correlations using the Spearman correlation.

Results: Significant differences were observed between *OPG* 950 genotype and IL10 (p=0.002), IL6 (p=0.004) concentrations. Significant positive correlations were found in non-BD group between IL10/ IL6 ($\rho = 0.736$, $p < 0.0001$) and in bone affected group between IL10/IL6 ($\rho = 0.606$, $p=0.013$) and IL10/ MIP1 α ($\rho = 0.064$, $p=0.011$). There were no significant differences in cytokine or genotype profiles related to presence or absence of BD. **Conclusions:** These preliminary data suggest that the *OPG* 950 genotype alters the IL6, IL10 plasma concentrations. Moreover the different correlations between cytokines in the different groups could indicate different bone signal activation pathways.

This work was partially financed by a grant from Genzyme a Sanofi Company.

Poster 34

The use of chitotriosidase as a parameter for the differentiation among sphingolipidoses.

Stumpfe K, Beck M, Mengel E.

Villa Metabolica, Center of Pediatric and Adolescence Medicine, MC JGU Mainz, Germany.

Introduction: Sphingolipidoses express themselves clinically with a wide range of symptoms but especially organomegaly (i.e. hepatosplenomegaly, splenomegaly or hepatomegaly and neurological signs) is observed. In order to differentiate between the variety of lysosomal storage diseases (LSD) chitotriosidase, a human chitinase is a rational biochemical marker with elevated activity in LSD which helps to narrow down the various differential diagnoses. Characteristic ranges could be determined among the sphingolipidoses. For this population we analyzed the feasibility of first step screening with chitotriosidase.

Methods: Using the method of Hollak et al 1994 data of 99 patients were collected and investigated in our laboratory. This single-centered, retrospective study describes the measurement of standard enzyme-assays of chitotriosidase before any therapeutic treatment for the last 6 years on children.

Results: The following diagnosis frequencies and chitotriosidase activities [nmol/ml/min] (median; 25th percentile; 75th percentile) were determined:

GD n= 30 (18564; 11335; 23673); SMD n= 17 (1087; 563; 1937); NPC n= 34 (646; 213; 998); M. Fabry n= 6 (178; 153; 175); CESD n=2 (249, 716); M. Faber n=2 (297, 3279); M. Krabbe n=1 (781); GM1 n= 3 (1013; 539,2646); Sialidosis n=1 (480); Galactosialidosis n= 4 (342, 734, 1948, 14066).

In GD, the activity of chitotriosidase was significantly higher compared to all other LSD. In the ultra-rare disease Sialidosis higher chitotriosidase activity was measured also in one case. SMD, GM1 and may Farber disease share nearly the same range of the enzyme's activity, as do NPC and M. Krabbe on a lower level.

Discussion: In the population studied, chitotriosidase activity can be sectioned in different compartments. Activities above 4000 nmol/ml/min were uniquely described for GD which implies this enzyme assay in narrowing down the differential diagnosis of the various LSD. Values around 1000 nmol/ml/min argue for diseases of SMD and GM1. NPC was located at a value of 600 nmol/ml/min. In a range of 170 - 300 nmol/ml/min just on the upper limit of normal diseases like M. Fabry and adult NPC were observed. M. Faber, Sialidosis and Galactosialidosis were measured in few patients a wide range of chitotriosidase activity level.

We conclude that chitotriosidase analysis is an important marker in differential work up of LSD. It is of assistance in identifying the most probable specific confirmatory enzyme assay in populations of patients with splenomegaly.

Poster 35

Plasmalogen levels and Gaucher disease: Further studies.

Moraitou M¹, Dimitriou E¹, Dekker N², Monopolis I³, Aerts H², Michelakakis H¹.

¹Department of Enzymology and Cellular Function and ³Department of Statistics, Institute of Child Health, Athens, Greece, ²Department of Medical Biochemistry, Academic Medical Center, University of Amsterdam, The Netherlands.

In Gaucher disease (GD) reduced red blood cell (RBC) plasmalogen levels, that showed a significant rise following treatment, have been reported. We further investigated plasmalogen levels in relation to glucosylceramide (GlcCer), ceramide (Cer), glucosylsphingosine (GlcSph) and malonyldialdehyde (MDA) levels in 27 GD patients and 13 controls. Plasmalogen levels were measured as their dimethylacetal derivatives (DMA) by GC in lipid extracts of RBC membranes and expressed as the ratios C_{16:0} DMA/C_{16:0} and C_{18:0} DMA/C_{18:0}. MDA using the TBA method, GlcCer and Cer using HPLC, were also quantified in RBC membrane extracts. Plasma GlcSph was quantified by LC-ESI-MS/MS. The results were analysed by non parametric tests. Both plasmalogen species were significantly lower in GD compared to controls ($p=0.009$ and $p<0.001$). There was no statistically significant difference between GD patients and controls with regard to GlcCer [GD: 6.5-113.4 pmol/10⁸ cells, median=35.2, n=24; controls: 16.7-46.8 pmol/10⁸ cells, median=24.9, n=12) and Cer levels [GD: 68-634 pmol/10⁸ cells, median=300, n=24; controls: 228-768 pmol/10⁸ cells, median=308, n=12]. However, in GD compared to controls the ratio GlcCer/Cer was significantly higher [0.073-0.231, median=0.116, n=24; 0.059-0.133, median=0.071, n=12; $p<0.001$] and GlcSph was more than 200-fold increased [28.1-812.0 nM, median=260.2, n=24; 0.8-2.7 nM, median=1.3, n=28]. MDA was significantly increased in GD patients compared to controls (0.7-10.6 nmol/10¹⁰ cells, median=4.5, n=27; 1.1-5.4, median=3.0, n=13; $p=0.019$). In both GD patients and controls a negative correlation between both plasmalogen species and GlcCer, Cer, GlcCer/Cer and GlcSph was observed, which was statistically significant only for the C_{16:0} DMA species in GD patients [GlcSph: $r_s=-0.420$, $p=0.041$; GlcCer: $r_s=-0.513$, $p=0.01$; Cer: $r_s=-0.452$, $p=0.027$]. A negative, not statistically significant correlation, was observed between MDA and both plasmalogen species in GD patients and only the C_{16:0} DMA species in controls. In conclusion, our results show a link between the reduced plasmalogen levels observed in GD with the lipid abnormalities characterizing the disorder, as well as an association with the increased oxidative stress observed in GD patients.

Poster 36

Chitotriosidase in Leishmania infection.

Dimitriou E¹, Moraitou M¹, Koutouzis E², Lianou D³, Syriopoulou V², Michelakakis H¹.

¹ Dept. Enzymology and Cellular Function, Institute of Child Health, Athens, ² 1st Dept. Pediatrics, University of Athens, ³ 1st Dept. Pediatrics, “Aghia Sophia” Children’s Hospital, Athens, Greece.

There are conflicting reports concerning the chitotriosidase plasma activity in patients with visceral Leishmaniasis (VL). In this study we investigated plasma chitotriosidase activity in patients with Leishmania infection on diagnosis and post treatment, as well as the presence of the 24bp duplication in the chitotriosidase gene. A total of 86 patients with VL due to *L. Infantum* and 894 controls from the general population were studied. In all patients, chitotriosidase plasma activity was measured on diagnosis and in 32 patients, chitotriosidase plasma activity was also measured at different time points post treatment. Chitotriosidase activity was assayed using the 4-methylumbelliferyl- β -D-N,N',N''-triacetylchitotrioside substrate. The 24bp duplication in the chitotriosidase gene was studied in 44 individuals with VL and 127 controls using PCR. In VL patients chitotriosidase plasma activity on diagnosis was 0-240 nmoles/ml/hr, median 48 (25th-75th centiles:22–83) with only two patients above the upper normal limit (normal range:0-150 nmoles/ml/hr, median 31 (25th -75th centiles:18-52). In the majority of patients with follow up measurements a decrease in the chitotriosidase plasma activity was observed 3-7 days after treatment (range:6%-60%). No consistent trend was observed 1 month later. Although 14% of the patients studied had zero chitotriosidase activity, homozygosity for the 24bp duplication was found in only 7%. In the control group the percentage of individuals with zero activity (7%) coincided with that of homozygous for the 24bp duplication (6%). Co-incubation of plasma samples from patients with zero activity with samples of known enzyme activity did not result in inhibition. Furthermore, assaying of chitotriosidase activity with 4-methylumbelliferyl-deoxychitobioside in a subgroup of the control individuals did not alter the zero status of the chitotriosidase activity. Overall, our results show that increased chitotriosidase activity is not a feature of Leishmania infection. The discrepancy between the results of DNA analysis and enzyme assays observed in patients and not in controls could possibly reflect either a genetic defect in chitotriosidase gene not described as yet or an effect of the parasite on the synthesis of chitotriosidase by its host. Long term studies and sequencing of the gene would be important.

Poster 37

Gaucher's disease as a cause of non-immune hydrops fetalis in the second trimester.

Gusina NB¹, Boysha AS², Zimovina TS¹, Novikova IV¹.

¹National Research and Applied Medicine Centre "Mother and Child", Minsk ²Mogilev Regional Centre for Medical Genetics, Mogilev, Republic of Belarus.

Objective: Gaucher's disease is an inborn error of glycosphingolipid metabolism caused by a deficiency of the lysosomal enzyme acid glucocerebrosidase (beta-glucosidase). The clinical spectrum of Gaucher's disease is very wide. The most severe clinical type, acute infantile type 2, can affect the fetus as early as the second trimester of pregnancy. This subtype, which can be named Gaucher's disease type 2 of fetal onset, manifests mainly as hydrops fetalis. Here we report on the early prenatal diagnosis of Gaucher's disease in a fetus with hydrops fetalis detected accidentally during the ordinary ultrasound examination in the second trimester.

Case report: The index case was 30-years old gravida 2, para 1. Her first pregnancy terminated at 10 weeks with spontaneous abortion. During the ultrasound examination at 20 weeks the fetus was found to have severe hydrops fetalis.

Before pregnancy termination at 21 weeks the diagnostic amniocentesis was performed and cultured amniocytes were assayed for chromosomal and metabolic causes of hydrops fetalis.

Methods: The diagnosis of Gaucher's disease was based on beta-glucosidase activity assay in cultured amniocytes. Chitotryosidase activity was determined in cell-free amniotic fluid by the micro modification of routine diagnostic method. The presence of the most common mutations and complex alleles of GBA1 gene: 84GG, IVS2+1, N370S, V394L, L444P, D409H, RecNciI and RecTL - was excluded in the fetal and parental DNA by allele-specific amplification.

Results: Almost total absence of beta-glucosidase activity in cultured amniocytes confirmed the diagnosis of Gaucher's disease in the fetus. Chitotryosidase activity in cell-free amniotic fluid was elevated (42 nmol/h/ml versus 2-4 normal). Pathological examination revealed the presence of abnormal "storage cells" in the fetal liver, spleen, heart and brain. The genetic counseling of the family was performed.

A year later the woman became pregnant again and the diagnostic chorionic villus biopsy was performed at 11.5 gestational weeks. Gaucher's disease was excluded by normal beta-glucosidase activity in chorionic tissue and cultured trofoblasts. During the ultrasound examination at 20 weeks the fetus had no pathological signs.

Conclusions: The case emphasizes the importance of the special biochemical and pathological examination of all fetuses with hydrops fetalis and also stresses that minimal and precocious echographic sings can be suggestive of Gaucher's and some other lysosomal storage diseases.

Poster 38

β -glucocerebrosidase (GBA) mutations in venezuelans patients with Gaucher disease. Preliminary results.

Molina Méndez K, Borjas L, Brown T, Sanchez Y, Zabala W, Cedeno R, Quintero J, Bracho D.

University of Zulia School of Medicine. Genetic Research Institute "Dr. Heber Villalobos Cabrera." Maracaibo, Venezuela.

Introduction: Gaucher disease (Online Mendelian Inheritance in Man #230800) is the most common autosomal recessive lysosomal storage disorder, which is caused by a β -glucocerebrosidase (GBA) deficiency. The definitive diagnosis of GD relies on demonstration of the deficit in enzyme activity and the primary genetic defect, from the molecular point of view, are the mutations in the GBA.

Aims: to determine the frequency of mutations N370S, L444P and 84insG in GD Venezuelans patients.

Methods: the molecular studies were performed on 24 unrelated patients, living in the west of the country, through the combination of the PCR and RFLP techniques, following the protocol described by Dr. Rosenberg in 2006 with certain modifications. Results: 7 patients (29.2%) were found to be compound heterozygotes N370S/L444P, 2 patients (8.3%) were homozygous for N370S, and this was repeated for 2 patients homozygous for L444P, only one patient (4.2%) had heterozygous mutation with 84insG N370S. The remaining patients 12 patients(50%) had the N370S mutation together with another unidentified.

Discussion: Through molecular techniques of PCR and RFLP was possible to identify the 75% mutant alleles (36/48), with the N370S mutation most commonly found (50%), followed in frequency of the L444P (22.9%); which coincides with that reported in other Caucasian populations. The PCR and RFLP are specific methodological strategies, reliable, efficient, reproducible and relatively quick to let you apply early treatment to those affected, provide appropriate genetic counseling to couples in need and provide a better quality of life for the affected population at risk and the Gaucher disease in Venezuela.

Data from this study are preliminary results and strategies are being developed based on the sequencing of the GBA gene with which it will be possible to identify 100% of the mutations present in affected individuals.

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Poster 39

Characterization of variants in the glucosylceramide synthase gene and their association with Gaucher disease phenotype.

Navascués J^{1,2}, Alfonso P^{1,3,4}, Andreu V^{1,4}, Navarro S⁵, Medina P⁵, Bolado-Carrancio A⁶, Irún P^{2,3,4}, Rodríguez-Rey JC⁶, España F⁵, Pocoví M^{2,3,4}, Giraldo P^{1,3,4,7}.

¹Traslational Research Unit. Miguel Servet University Hospital. Zaragoza. Spain.

²Department of Biochemistry and Cellular and Molecular Biology. University of Zaragoza. Zaragoza. Spain.

³Biomedical Network Research Center on Rare Diseases (CIBERER), ISCIII. Valencia. Spain.

⁴Aragon Health Sciences Institute (I+CS). Zaragoza. Spain.

⁵Research Center, La Fe University Hospital. Valencia. Spain.

⁶Department of Molecular Biology. University of Cantabria and Instituto de Formación e Investigación Marqués de Valdecilla (IFIMAV). Santander. Spain.

⁷Service of Hematology. Miguel Servet University Hospital. Zaragoza. Spain.

Introduction. The extreme phenotypic variability of Gaucher Disease (GD) patients is not explained by glucocerebrosidase (*GBAI*) gene mutations. Previously, it was proposed that genetic modifiers might influence the GD phenotype. Glucosylceramide synthase gene (*UGCG*), which encodes protein involved in the synthesis of glucosylceramide, could be a candidate modifier gene.

Aim. To determine whether *UGCG* polymorphisms affect the GD severity.

Methods. We analysed the frequency of seven *UGCG* variants in 290 control individuals and 112 GD patients. The association of the Severity Score Index (SSI) with the *UGCG* polymorphisms was studied in two GD patient subgroups according to their *GBAI* genotype.

Also, we carried out a detailed analysis of two *UGCG* variants, g(-231)₋(-222)ins10 and g.148A>G. Using EMSA analysis, we investigated if the presence of the g(-231)₋(-222)ins10 and g.148A>G polymorphisms might influence the interaction with nuclear proteins. First, we constructed pGL3-Basic-*UGCG* plasmids carrying three different haplotypes: [=] (wild type), [g(-231)₋(-222)ins10] and [g.148G], and then studied their promoter activities by luciferase assays.

Results. Five *UGCG* variants in heterozygosity were significantly associated with SSI values: g(-295)C>T, g(-231)₋(-222)ins10, g.148A>G, g.166A>T (RefSeq 3780519) and g.34991A>G (RefSeq 7850023). Two of them, g(-295)C>T and g.166A>T, were found in linkage disequilibrium.

The heterozygous patients with g.[(-231)₋(-222)ins10(+)]148G haplotype, showed a significant decrease of SSI values in respect to the patients carrying only one of them, for total GD patients and [N370S]+[L444P] subgroup.

EMSA analysis showed an altered pattern of nuclear protein binding for the g(-231)₋(-222)ins10 probe and an increase of protein binding ability of the G allele at g.148 position.

In silico studies suggested that the insertion generates a new binding site for EGFR-specific transcription factor and that the g.148A>G variant could affect the binding site for AP-2 transcription factor.

The promoter activity of the g.148G haplotype decreased significantly with regard to the wild type activity in HepG2 and COS-7 cells (-34% and -23%, respectively).

Discussion. Our study indicate that the g(-231)₋(-222)ins10 and g.148A>G variants could be modifier factors on the GD severity and, in part, explain the phenotypic variability of the disease.

Poster 40

Changes in chitotriosidase activity in Gaucher disease during treatment in relation to the dup24 and G102S polymorphisms.

Irún P^{1,2}, Alfonso P^{1,3}, Aznarez S², Giraldo P^{1,3,4}, Pocovi M^{1,2,3}.

¹Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Zaragoza, Spain. ²Biochemistry and Molecular and Cellular Biology Department, Zaragoza University, Spain. ³Instituto de Investigación Sanitaria de Aragón (IIS), Spain. ⁴Haematology Department, Miguel Servet University Hospital, Zaragoza, Spain.

Human plasma chitotriosidase activity (CT), a biomarker for Gaucher disease (GD), vary in general population due to polymorphisms in the CHIT1 gene. Our aim was to analyze the frequency of c.1049_1072dup24 and p.G102S polymorphisms and their influence on plasma CT and in CT changes during the first year on enzymatic replacement therapy (ERT; mean doses 36±12 IU/Kg, range: 15-60 IU/kg/eow) in Type I GD patients. CT genotypes were analyzed by PCR, gel electrophoresis and restriction isotyping in a total of 246 patients from Spanish Gaucher Disease Registry. Plasma CT activity was analyzed before and after a year on ERT using 4-methylumbelliferyl-β-D-N,N',N''-triacetylchitotriose at nonsaturating concentrations. As a result we found allelic frequencies for dup24 and G102S in our cohort of 0.22 and 0.25 respectively, 4% of patients were homozygous (HOMOdup) and 37% heterozygous (HETdup) for dup24 and 8% homozygous (HOMO102) and 37% heterozygous (HET102) for G102S. GD patients were classified in two groups: negative for c.1049_1072dup24 (NEGdup) and negative for G102S (NEG102). We have found significant differences in CT before ERT between HETdup versus NEGdup and also between HET102 and HOMO102 when compared to NEG102 (see Table). No differences in the percentage of CT variation during the first year on ERT were observed in relation with CHIT1 genotype for both polymorphisms (see Table).

NEG102				NEGdup			
CT at diagnosis (nmol/mL.hr)				CT at diagnosis (nmol/mL.hr)			
	N	Mean±SD	p		N	Mean±SD	p
NEGdup	62	15,600±12,346		NEG102	62	15,600±12,346	
HETdup	54	6,366±4,120	0.001	HET102	60	10,300±7,809	0.024
				HOMO102	15	7,749±3,543	0.037
CT change after 1 year ERT (%)				CT change after 1 year on ERT (%)			
	N	Mean±SD	p		N	Mean±SD	p
NEGdup	34	78±14		NEG102	34	78±14	
HETdup	32	77±16	0.850	HET102	35	70±20	0.067
				HOMO102	7	72±23	0.389

In conclusion, CT activity in naïve patients depend on the presence/absence of c.1049_1072dup24 and p.G102S polymorphisms in CHIT1. However, the percentage of CT decrease after a year on ERT is independent of the presence of c.1049_1072dup24 as well as independent of the presence of p.G102S.

Poster 41

Clinical diagnosis of Gaucher (GD) type 2 : A real challenge.

Bracho A¹, Cedeño R¹, Chong J¹, Morales A¹, Borjas L¹, Urdaneta K¹, Gonzalez M².

¹ Unidad de Genética Médica de La Universidad del Zulia, ² Hospital Chiquinquirá de Maracaibo. Venezuela

Introduction : Gaucher disease (GD) type 2 or acute neuronopathic is a very severe and progressive form of GD, with rapidly fatal neurological symptomatology. It has an estimated frequency of 1 in 100,000 to 500,000 live births. Children have normal development until 3 to 6 months of age and they usually die before 2 years.

Objective : to perform through a case report, the clinical diagnosis process of GD type 2.

Case report : A female infant of 14 months of age, born to a non-consanguineous venezuelan couple with an uneventful perinatal course and normal psychomotor development until 6 months. She had hospitalization at 7 and 10 months of age with a diagnosis of pneumonia, anemia and seizure syndrome. At 6 months of age she subsequently developed feeding difficulties and had regression of motor milestones and at 14 months of age she had the third pediatric emergency consultation because breathing difficulties. On examination she weighed 5000grs, height: 67 cm, CC: 40 cms. Generally poor condition, pale skin mucosa, low adiposity, gaze palsy, swallowing impairment, breath sounds with rhonchi audible and bullous, chest indrawing, abdominal distension, neurological features: generalized hypertonia, oculomotor apraxia, hyperextension of the neck. Laboratory Findings: moderate anemia and thrombocytopenia, urine culture, stool culture and blood culture: negative; Abdominal Echogram: hepatosplenomegaly mild to moderate, diffuse non-specific Chest XR: Pneumonia right. During her hospitalizations she had several seizures and she had been assessed by a multidisciplinary team, that taking into account the background and torpid evolution, as well as ultrasound and laboratory findings, coupled with neurological impairment, decided to discard GD type 2. Blood sample was taken to determine the enzymatic activity of β glucosidase (BGA), which reduced reported (0.1 mmol / lh), with elevation of chitotriosidase activity (1622.27 mmol / lh), results consistent with GD. The patient died at 23 days of hospitalization.

Discussion : GD type 2 must be regarded as a pediatric disease which onset in childhood is predictive of a progressive and severe phenotype, with the presence of neurological symptoms, and the patient must be conducted by a multidisciplinary team. The purpose of this paper is affront an challenge in clinical genetics and pediatrics insights of managements for this condition including implications for genetic counseling.

Poster 42

Pregnancy revealing Gaucher disease : Case report.

Chaabane K, Zouari F, Rekik M, Kebaili S, Lehiani I, Ben Hmid R, Amouri H, Guermazi M.

Hedi Chaker Academic Hospital, Gynecology obstetric department, Sfax, Tunisia.

Introduction : Gaucher disease (GD), characterized by deficient acid β -glucosidase activity, is the most common lysosomal storage disorder. The disease is progressive with manifestations that include anemia, thrombocytopenia, organomegaly and bone disease. Pregnancy can exacerbate these manifestations, including the risk of complications during pregnancy, delivery and postpartum. Pregnancy in GD has several risks and an appropriate follow-up should be planned at the beginning of pregnancy in these patients

Aim : To optimize patient care around pregnancy, delivery and the postpartum period, and alert obstetricians to the possible complications of pregnancy and delivery in GD.

Methods : case report.

Case report

A 30 year-old female patient, primiparous was hospitalised for the investigation of massive splenomegaly, thrombocytopenia and abdominal pain at 34th week. The laboratory findings on admission, were as follows: hemoglobin 9 g/dl, platelet count: 73.000/mm³, serum transaminases: SGOT: 28 U / dl, SGPT: 18U /dl. The ultrasonographic size of her spleen was 16 ×7 cm. Clinical examination noted a normal blood pressure and no proteinuria. The diagnosis of GD was based on the presence of characteristic Gaucher cells in a bone marrow aspiration (40–50% infiltration) and was established by the estimation of white bloodcell b-glucocerebrosidase (4 nmol / mg Protein / h, normal range: 6–23 nmol / mg Protein / h). Blood transfusions were only required at deliverie carried out on the 36th week by elective cesarean section. Neonates (male) was normal, weighing 2400 g. No postoperative complications were observed.

Discussion : For a long time pregnancy and Gaucher disease were considered to be incompatible and pregnancies were discouraged in women with the disorder. Pregnancy concurrent with GD has several risks including an increased severity of anaemia and thrombocytopenia, that can potentiate post partum bleeding, significant increase in organomegaly, worsening of skeletal manifestations and possibly an increased spontaneous abortion rate. Evolution of Gaucher Disease (GD) during pregnancy is heterogeneous depending mainly on the severity of disease at the beginning of pregnancy. As disease was controlled, mothers are more likely to experience uncomplicated pregnancies and deliveries.

Poster 43

Increased bone resorption due to high serum C-C motif Ligand-3 (CCL-3) is present in patients with non-neuronopathic Gaucher disease.

Komninaka V¹, Marinakis T², Christoulas D³, Repa K⁴, Kolomodi D⁵, Mitropoulou M⁵, Gkatzamanidou M³, Papatheodorou A³, Michelakakis H⁶, Voskaridou E¹, Dimopoulos MA³, Terpos E³.

¹Department of Thalassemia, Laikon General Hospital, Athens, Greece; ²Department of Hematology, "Georgios Gennimatas" General Hospital, Athens, Greece; ³Department of Clinical Therapeutics, University of Athens School of Medicine, Athens, Greece; ⁴Department of Hematology, Polykliniki General Hospital, Athens, Greece; ⁵Department of Radiology, St Savvas Oncology Hospital, Athens, Greece; ⁶Department of Enzymology and Cellular Function Institute of Child Health, Athens, Greece.

Introduction : Gaucher disease (GD) is characterized by skeletal manifestations, including osteopenia and osteoporosis. Low bone mass is due to enhanced bone resorption mediated mainly by macrophage-derived factors, such as the C-C motif ligand 3 (CCL-3) chemokine.

Aims : The aim of this study was to evaluate the clinical and biochemical characteristics of bone involvement in patients with non-neuronopathic GD.

Methods : We studied 27 adult patients (15M/12F, median age 44.5 years). DXA scan of the lumbar spine (L) and femoral neck and MRI of the femoral bones were performed in all patients. We also evaluated the circulating levels of CCL-3 along with two sensitive serum markers of bone resorption: C-telopeptide of collagen type-1 (CTX) and tartrate resistant acid phosphatase isoform-5b (TRACP-5b). The above molecules were also evaluated in 25 healthy, gender- and age-matched controls, and in all patients 6 months post initiation of replacement therapy.

Results : Twelve patients (46%) had bone pain, while 5 patients had restrictions in free movement. The majority of the patients (91%) had elevated chitotriosidase levels (median: 1647 nM/ml/hr, range: 147-18880 nM/ml/hr; UNL: 150 nM/ml/hr) indicating active disease, while 25% of the patients had abnormally high levels of acid phosphatase. Six (22%) patients had osteoporosis, 18 (66%) patients had osteopenia and only 3 patients had normal DXA scan. All but one patient had abnormal MRI findings: 3 (11%) had grade I MRI abnormalities, 18 (66%) grade II, 3 (11%) grade III and 2 (7%) grade IV. Erlenmeyer flask sign was observed in 23 (85%) patients. Patients with osteopenia or osteoporosis had elevated chitotriosidase levels compared to others ($p=0.035$). Patients with advanced MRI disease (grades III/IV) had elevated levels of acid phosphatase compared to those with grade I/II ($p=0.011$).

At study initiation, GD patients had elevated TRACP-5b and CCL-3 compared to controls ($p<0.001$). There was a positive correlation between TRACP-5b and chitotriosidase levels ($p=0.017$) as well as between TRACP-5b and CCL-3 levels ($r=0.512$, $p=0.01$). Six months post replacement therapy TRACP-5b ($p=0.011$) and CCL-3 ($p=0.01$) were significantly reduced.

Discussion : Our study suggests that patients with non-neuronopathic GD have abnormal MRI findings in the femoral bones and increased incidence of bone loss, which is at least partially due to increased CCL-3. Replacement therapy reduces abnormal osteoclast function.

Poster 44

Chaperone effect of several products, mostly aminocyclitols, on mutated GBAs from Gaucher patients' fibroblasts.

Díaz L^{a,b}, Serra J^c, Sánchez-Ollé G^c, Grinberg D^c, Michelakakis H^d, Mavridou I^d, Delgado A^{a,b}, Bujons J^b, Casas J^b, Vilageliu L^c.

^a Unitat de Química Farmacèutica (Unitat Associada al CSIC), Facultat de Farmàcia, Universitat de Barcelona. ^b Research Unit on BioActive Molecules (RUBAM), Departament de Química Biomèdica, Institut de Química Avançada de Catalunya, CSIC, Barcelona, Spain. ^c Departament de Genètica, Universitat de Barcelona; IBUB; CIBERER, Barcelona, Spain. ^d Department of Enzymology and Cellular Function, Institute of Child Health, Athens, Greece.

Gaucher disease is a lysosomal storage disorder due to an inherited deficiency of the lysosomal enzyme glucocerebrosidase (GBA). It results in the accumulation of glucosylceramide in cells of the reticuloendothelial system and causes multisystemic manifestations, including hepatosplenomegaly, skeletal lesions and, sometimes, neurological involvement. The limited efficacy of the current treatments has led to the development of new strategies, including the use of chemical chaperones. These are small molecules, generally competitive inhibitors of the target enzyme, that at sub-inhibitory concentrations may induce the proper folding and trafficking of the mutated enzyme resulting in a concomitant increase in the residual activity.

The aim of this work is to report on the ability of eleven compounds to increase the residual GBA activity on fibroblasts from six Gaucher patients bearing different genotypes. Nine of the products are new aminocyclitols (LD family), and the remaining two are *N*-(*n*-nonyl)-deoxynojirimycin (NN-DNJ) and isofagomine (IFG).

Fibroblasts were cultured in 24-well plates during 6 days either with or without the compound to be assayed. Then, the GBA assay was performed.

The two genotypes that include mutation G202R showed the best chaperone response for most of the compounds, being very high for the isofagomine. Compound LD159, and also LD25 showed a reasonable effect on the GBA activity of the N370S/N370S fibroblasts. On L444P enzyme only a few products seemed to be slightly active. Currently, we are undertaking lipidomic assays in order to analyze the levels of glucosylceramide in those cases where an increase of the GBA activity were observed.

ACKNOWLEDGMENTS

PARTENER OF THE CONGRESS

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Support for the European Working Group on Gaucher Disease
2012 meeting

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