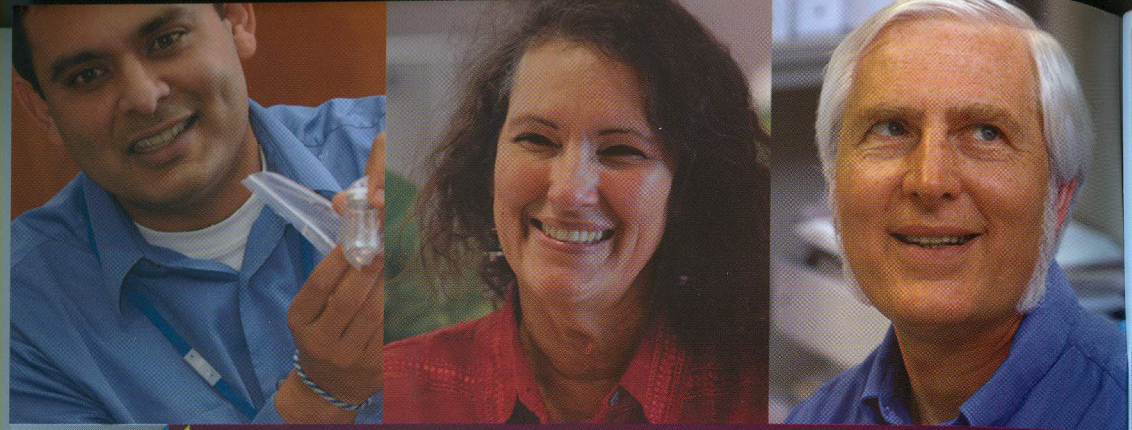
8th European Working Group on Gaucher Disease (EWGGD) Meeting

June 4-7, 2008
Budapest, Hungary

FINAL PROGRAMME AND ABSTRACT BOOK

www.ewggd2008.com



Shire HGT–
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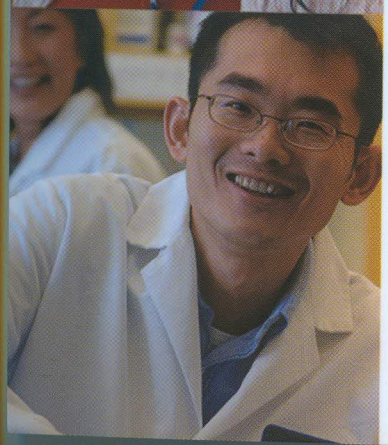

Shire HGT is proud to support the 8th European Working Group on Gaucher Disease

The people of Shire Human Genetic Therapies are committed to developing novel therapies to treat life-threatening genetic diseases.

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Human Genetic Therapies

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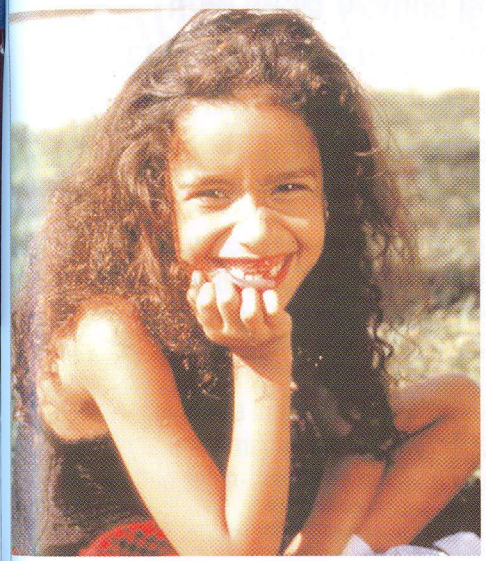

Lysosomal Storage Disorders

Renal Diseases

Orthopaedics

Oncology/Endocrinology

Transplant/Immune Diseases

Biosurgical Specialties

Genetics/Diagnostics

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8TH EUROPEAN WORKING GROUP ON GAUCHER DISEASE (EWGGD) MEETING

FINAL PROGRAMME & ABSTRACT BOOK

www.ewggd2008.com

June 4–7, 2008
Danubius Health Spa Resort Margitsziget
Budapest, Hungary

CONTENTS

8TH EUROPEAN WORKING GROUP ON
GAUCHER DISEASE (EWGGD) MEETING
JUNE 4–7, 2008

Welcome to Budapest

On behalf of the Scientific Committee and the Local Organizing Committee of the 8th European Working Group on Gaucher Disease (EWGGD) Meeting we are delighted to welcome you to Budapest.

We are honoured to be the host of the Meeting and to provide a forum for delegates to learn together, share experiences, strengthen links and explore collaborative approaches in the field of Gaucher disease.

Budapest is a dynamic and vibrant city offering a large scale of cultural and natural beauties. This is the only capital in the world where more than 100 hot thermal springs feed a wide range of baths.

Budapest is awaiting you with its mixture of Eastern and Western cultural tradition, beautiful spas, and the gala dinner – cruising on the Danube – will get you acquainted with the splendid panorama of the two banks of the river.

László Maródi
Chairman
Local Organizing Committee

Hans Aerts
Chairman
EWGGD

Timothy Cox
Vice-chairman
EWGGD

Scientific Committee

Hans Aerts, University of Amsterdam, Amsterdam
Bruno Bembi, Centre for Rare Disorders, Udine
Tim Cox, University of Cambridge, Cambridge
Carla Hollak, University of Amsterdam, Amsterdam
László Maródi, University of Debrecen, Debrecen

Scientific Information

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E-mail: lmarodi@dote.hu
Phone: +36 52 416 841
Fax: + 36 52 430 323

Local Organising Committee

László Maródi, University of Debrecen, Hungary – chair of Committee
Melinda Erdős, University of Debrecen, Debrecen
Gabriella Kürti, University of Debrecen, Debrecen
Judit Tóth, University of Debrecen, Debrecen

Congress Office

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Fax: + 36 1 299 0187

8TH EUROPEAN WORKING GROUP ON
GAUCHER DISEASE (EWGGD) MEETING
JUNE 4–7, 2008

Invited Speakers

Hans Aerts, University of Amsterdam, Amsterdam

Timothy Cox, University of Cambridge, Cambridge

Deborah Elstein, Shaare Zedek Medical Centre, Jerusalem

Pilar Giraldo, University of Zaragoza, Zaragoza

Jack Goldblatt, University of Melbourne, Melbourne

Carla Hollak, University of Amsterdam, Amsterdam

Mia Horowitz, Tel Aviv University, Tel Aviv

Derralynn Hughes, Royal Free & University College Medical School, London

Stefan Karlsson, Lund University, Lund

György Kosztolányi, University of Pécs, Pécs

Mirjam Langeveld, University of Amsterdam, Amsterdam

Atul Mehta, Royal Free Hospital and University College of London, London

Pramod Mistry, Yale University School of Medicine, New Haven, CT

Gregory Pastores, New York University School of Medicine, New York, NY

Paul Saftig, Christian-Albrechts University, Kiel

Anna Tylki-Szymanska, Children's Memorial Health Institute, Warsaw

Ashok Vellodi, Great Ormond Street Hospital for Children, London

Stephan vom Dahl, Heinrich Heine University, Düsseldorf

Ari Zimran, Shaare Zedek Medical Centre, Jerusalem

General Information

Congress venue
The meeting takes place at the Danubius Health Spa Resort Margitsziget, linked with an indoor corridor to the Danubius Grand Hotel Margitsziget. Except for the welcome reception that is located at the Széchenyi restaurant of the Grand Hotel, all activities related to the meeting take place at the Health Spa Resort.

Registration
Registration desk is situated in the lobby of the congress hotel.
Registration desk opening hours:
Wednesday, June 4, 2008: 13:00-20:00 hrs
Thursday, June 5, 2008: 08:00-19:00 hrs
Friday, June 6, 2008: 08:30-19:00 hrs
Saturday, June 7, 2008: 08:30-13:00 hrs

Registration items
Your conference registration includes access to the scientific programmes and exhibition area, congress bag, final programme and abstract book, coffee and soft drink during breaks, welcome reception on Wednesday evening, buffet lunch on Thursday, Friday, Saturday and gala dinner on Friday evening.
All catering services are available against tickets received at the registration desk.

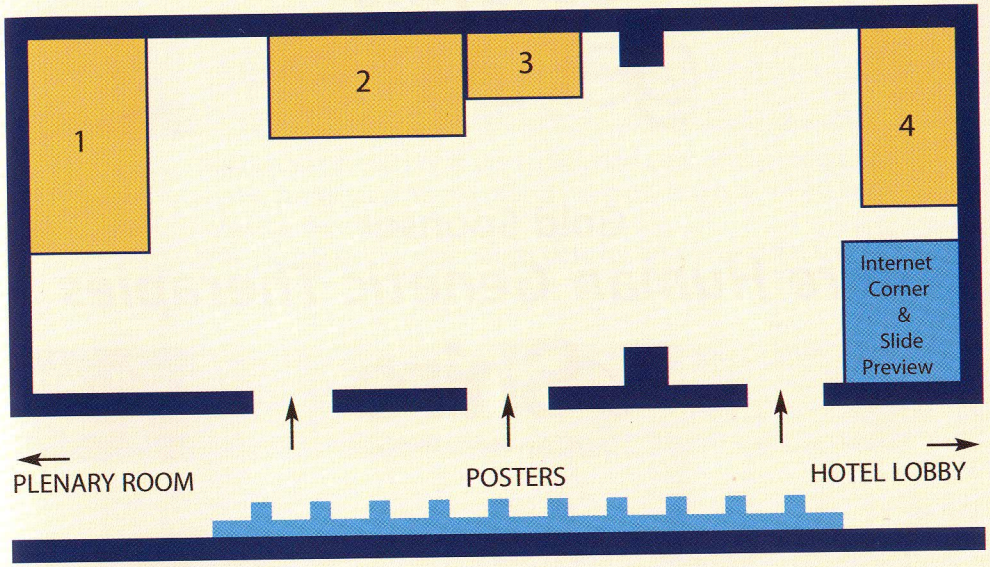
Meeting rooms
Plenary room: Star Audotorium on floor -1
Exhibition and coffee break area: Jasmine room on floor 0
Posters: corridor in front of Jasmine room on floor 0
Internet corner and slide preview area: Jasmine room on floor 0
Lunch: Restaurant Platán on floor 0

Posters
Posters are located on floor 0 in the congress hotel, on the corridor in front of room Jasmine. Delegates may set up their posters from 15:00 hrs on Wednesday, June 4, 2008 and leave them for the entire duration of the meeting. Please put your poster on the board bearing your name and the title of your abstract on. Technical assistance will be provided on spot. Posters shall be removed right after the official closing of the meeting on Saturday, June 7, 2008. The poster boards will be dismantled at 14:00 hrs on that day and any poster left on the board will be automatically discarded.
You are kindly requested to be standing near your poster during the poster viewing period, which is Saturday, June 7, 2008 between 11:00-11:30 hrs to answer any questions that the poster reviewers and delegates may have.

Oral presentations – slide preview area
Your presentation shall be loaded with the help of our technician at the slide preview area located in the room Jasmine on floor 0 in the congress centre. Presentations taking place before noon should be saved by 09:00 hrs, while presentations in the afternoon should be saved by 13:30 hrs on the day of your presentation.

8TH EUROPEAN WORKING GROUP ON
GAUCHER DISEASE (EWGGD) MEETING
JUNE 4–7, 2008

Exhibition Area Floorplan



EXHIBITORS

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Social Programmes

10.00–14.00
Registration Desk
Floor 0

BUDAPEST CITY TOUR (OPTIONAL)

Meeting at 10:00 hrs at the registration desk of the congress hotel. Arrival back to the official congress hotels at 14:00 hrs.
Meals not included.
Please contact the registration desk for on-site registration.

19.00–19.30
Star Auditorium
Floor -1

OPENING CONCERT

appr.
19.45–22.00
Restaurant
Széchenyi
Grand Hotel
Floor 0

WELCOME RECEPTION

Informal opening buffet reception on the terrace (provided weather permits) of the Széchenyi Restaurant of the Grand Hotel. Delegates are kindly requested to walk through to the Grand Hotel right after the finishing of the opening concert.

19.30
Registration Desk
Floor 0

HUNGARIAN DINNER (OPTIONAL)

Meeting at 19:30 hrs at the registration desk of the congress hotel.
Bus transfer will be arranged to the dinner venue and back to the official congress hotels.
Venue: Kárpátia Restaurant
Address: H-1053 Budapest, Ferenciek tere 7-8.
Please contact the registration desk for on-site registration.

14.00–18.00
Registration Desk
Floor 0

BUDAPEST CITY TOUR (OPTIONAL)

Meeting at 14:00 hrs at the registration desk of the congress venue. Arrival back to the congress venue at 18:00 hrs. Meals not included.
Please contact the registration desk for on-site registration.

19.30
Registration Desk
Floor 0

GALA DINNER

Meeting at 19:30 hrs at the registration desk of the congress hotel.
Dinner on the Europa Boat. The boat will be waiting for us close to the hotel.
Boat leaves for cruising at 20:15 hrs the latest.

10.00–15.00
Registration Desk
Floor 0

EXCURSION TO GÖDÖLLŐ (OPTIONAL)

Programme cancelled, kindly contact the registration desk for reimbursement.

Saturday

List of Poster Presentations

P 1	Common variants in the glucosylceramide synthase gene are associated with disease severity in Gaucher disease patients	Pilar Alfonso	University of Zaragoza, Zaragoza
P 2	Identification and functional characterization of four novel mutant alleles causing Gaucher disease.	Bruno Bembi	Centre for Rare Disorders, Udine
P 3	The neurological manifestations of Gaucher's disease type 1: The French Observatoire on Gaucher Disease (FROG)	Patrick Cherin	Hospital Pitie-Salpetriere, Paris
P 4	Conflicting results in leucocytes and fibroblasts in two unusual cases of Gaucher disease	Heather Church	Royal Manchester Children's Hospital, Manchester
P 5	Serum chitotriosidase activity in heterozygotes for chitotriosidase deficiency (24 bp duplication) Gaucher patients on enzyme replacement therapy	Barbara Czartoryska	Institute of Psychiatry and Neurology, Warsaw
P 6	Response to enzyme replacement therapy with velaglucerase alfa,gene-activated human glucocerebrosidase, in a patient with type 1 Gaucher disease (GD)	Gonzalez Derlis	Sanatorio Espanol, Asuncion
P 7	A new type I Gaucher disease severity score index for phenotypic classification and evaluation of responses to treatment	Maja Di Rocco	Gaslini Institute, Genoa
P 8	Postimmunization antibody responses to polysaccharide antigens in splenectomized and non-splenectomized patients with type 1 Gaucher disease	Melinda Erdős	University of Debrecen Medical and Health Science Center, Debrecen
P 9	Development of a novel orally administered non-viral gene therapy for Gaucher disease using DNA nanoplexes encapsulated in yeast cell wall particles	Edward Ginns	University of Massachusetts Medical School, Massachusetts
P 10	Home therapy is safe for GA-GCB intravenous enzyme replacement therapy in patients with type 1 Gaucher disease	Yael Greenberg	Shaare Zedek Medical Center, Jerusalem
P 11	Gaucher disease: A model to study the role of glycosphingolipids in atherosclerosis	Johanna Groener	University of Amsterdam, Amsterdam
P 12	Persistent bone disease in adult type 1 Gaucher disease despite increasing doses of enzyme replacement therapy	Carla Hollak	University of Amsterdam, Amsterdam
P 13	Post-marketing surveillance update for miglustat in type 1 Gaucher disease (GD1)	Carla Hollak	University of Amsterdam, Amsterdam
P 14	Ambroxol is a Unique Pharmacological Chaperone for Human ²-Glucocerebrosidase and a Potential Treatment for Gaucher Disease	Pedro Huertas	Massachusetts General Hospital & ExSAR Corporation, Boston, MA

P 15	Low HDL cholesterol levels in type I Gaucher disease do not lead to an increased risk of cardiovascular disease	Mirjam Langeveld	University of Amsterdam, Amsterdam
P 16	Long term efficacy and safety of miglustat therapy in type 1 Gaucher disease. ZAGAL study	Paz Latre	FEETEG, Zaragoza
P 17	Inefficacy of drilling in early stages of osteonecrosis in Gaucher disease	Ehud Lebel	Shaare Zedek Medical Center, Jerusalem
P 18	Differential in vitro responses in inflammatory and immune cytokine production elicited by enzyme replacement therapies for Gaucher disease	Paolo Martini	Shire Human Genetic Therapies, Lexington
P 19	A multicenter, randomized, dose frequency study of the safety and efficacy of Cerezyme® infusions every 4 weeks versus every 2 weeks in the maintenance therapy of patients with type 1 Gaucher disease	Atul Mehta	Royal Free and University College Medical School, London
P 20	Progressive kyphoscoliosis in patients with chronic neuronopathic Gaucher disease	Eugen Mengel	Universitäts-Kinderklinik Mainz, Villa metabolica
P 21	Plasmalogen levels in Gaucher disease	Helen Michelakakis	Institute of Child Health, Athens
P 22	Time interval between diagnosis of Type 1 Gaucher Disease and initiation of Enzyme Therapy and splenectomy are determinants of avascular necrosis	Pramod Mistry	Yale University School of Medicine, New Haven
P 23	Bone marrow transplantation for acute myeloid leukemia from donor with Gaucher disease followed by Enzyme Replacement Therapy (ERT)	Mirando Mrsic	University Hospital Zagreb, Zagreb
P 24	Enhanced abundance and processing of Cathepsin S: a potential biomarker of Gaucher disease	Elena Pavlova	University of Cambridge, Cambridge
P 25	Biomarkers of Avascular Necrosis in Gaucher Disease	Elena Pavlova	University of Cambridge, Cambridge
P 26	Epileptic encephalopathy in patients with chronic neuronopathic Gaucher disease	Jörg Reinke	Universitäts-Kinderklinik Mainz, Villa metabolica
P 27	Bone densitometry usefully in the evaluation of bone disease in type 1 Gaucher patients. A preliminary comparative study	Mercedes Roca	Instituto Aragonés de Ciiencias de la Salud, Zaragoza
P 28	Validation of saccadic latency as a biomarker of cerebral injury in lysosomal storage disorders	Jonathan Roos	University of Cambridge, Cambridge
P 29	Hematological malignancies in type I Gaucher disease: Diagnosis and treatment challenge	Hanna Rosenbaum	Rambam Medical Center, Haifa

8TH EUROPEAN WORKING GROUP ON GAUCHER DISEASE (EWGGD) MEETING JUNE 4–7, 2008

P 30	Immune thrombocytopenia in type I Gaucher disease	Hanna Rosenbaum	Rambam Medical Center, Haifa
P 31	Chaperone effect of several iminosugars and aminocyclitols on mutated glucocerebrosidases as a possible therapeutic approach for Gaucher disease	Gessami Sanchez-Olle	University of Barcelona, Barcelona
P 32	Management of patients with Gaucher's disease (GD) in a French centre	Jérome Stirnemann	University Paris North, Paris
P 33	Long -term bone effect of enzyme replacement therap (ERT) in two splenectomizedchildren with type 1 Gaucher disease(GD)	Judit Tóth	University of Debrecen Medical and Health Science Center, Debrecen
P 34	Modelling the evolution of biomarkers under treatment in Gaucher disease	Corine Vincent	Inserm U738 - Université Paris 7, Paris
P 35	A report from the International Collaborative Gaucher Group (ICGG) Gaucher registry	Stephan vom Dahl	St. Franziskus-Hospital, Cologne
P 36	Development of a disease severity scoring system for type 1 Gaucher disease	Stephan vom Dahl	St. Franziskus-Hospital, Cologne
P 37	A phase 2 clinical trial of the parmacological chaperone at2101 for the treatment of Gaucher disease	Neal J. Weinreb	Research Foundation for Lysosomal Storage Diseases, Inc., Coral Springs
P 38	An improved high-throughput multiplex enzyme assay to screen for lysosomal storage disorders in dried blood spots	Kate Zhang (presenter: Tim Edmunds)	Genzyme Corporation, Framingham
P 39	High-throughput screening assay of Gaucher disorder by dried blood spots	Kate Zhang (presenter: Tim Edmunds)	Genzyme Corporation, Framingham
P 40	Up to 42-Months on treatment: Open-label phase I/II long-term study of Enzyme Replacement Therapy (ERT) with velaglucerase alfa in patients with type 1 Gaucher disease	Ari Zimran	Shaare Zedek Medical Center, Jerusalem
P 41	Management of reproductive events in females with Gaucher disease	Ari Zimran	Shaare Zedek Medical Center, Jerusalem

Final Programme

13.00–20.00
Registration Desk
Floor 0

REGISTRATION

18.00–19.00
Star Auditorium
Floor -1

INVITED LECTURE 1

Chair: *László Maródi,* University of Debrecen, Debrecen

LIMP-2 is a receptor for lysosomal mannose-6-phosphate-independent targeting of ß-Glucocerebrosidase
Paul Saftig, Christian-Albrechts University, Kiel

19.00–19.30
Star Auditorium
Floor -1

OPENING CONCERT

Performed by the *Budapest Saxophone Quartet* and *Ingrid Kertesi,* solist of the Hungarian State Opera House.

appr.
19.45–22.00
Restaurant
Széchenyi
Grand Hotel
Floor 0

WELCOME RECEPTION

08.00–19.00
Registration Desk
Floor 0

REGISTRATION

09.00–10.40
Star Auditorium
Floor -1

SESSION 1
Pathophysiology and manifestations of type 1 Gaucher disease

Chairs: *Hanna Rosenbaum,* Rambam Medical Centre, Haifa
Timothy Cox, University of Cambridge, Cambridge

09.00–09.20 **Pathophysiology: liver and lung manifestations**
Pramod Mistry, Yale University School of Medicine, New Haven, CT

09.20–09.30 **A new type I Gaucher disease severity score index for phenotypic classification and evaluation of responses to treatment**
Maja Di Rocco, University of Genoa, Genoa

09.30–09.40 **Development of a disease severity scoring system for type 1 Gaucher disease**
Stephan vom Dahl, St. Franziskus-Hospital, Academic Teaching Hospital, University of Cologne, Cologne

09.40–09.55 **Joint discussion**

09.55–10.05 **Saposin C deficiency**
Anna Tylki-Szymanska, Children's Memorial Health Institute, Warsaw

10.05–10.15 **Insulin resistance in Gaucher disease**
Mirjam Langeveld, University of Amsterdam, Amsterdam

10.15–10.25 **Gaucher disease and cancer**
Derralynn Hughes, Royal Free & University College Medical School, London

10.25–10.40 **Joint discussion**

10.40–11.00 *Room Jasmine Floor 0*

COFFEE BREAK

11.00–12.55 *Star Auditorium Floor -1*

SESSION 2
Enzyme replacement therapy

Chairs: *Ashok Vellodi,* Great Ormond Street Hospital for Children, London
Pilar Giraldo, University of Zaragoza, Zaragoza

11.00–11.15 **Dose-response relationships in Gaucher disease**
Stephan vom Dahl, St. Franziskus-Hospital, Academic Teaching Hospital, University of Cologne, Cologne

11.15–11.30 **Monitoring skeletal response**
Jack Goldblatt, University of Melbourne, Melbourne

11.30–11.45 **A multicenter, randomized, dose frequency study of the safety and efficacy of Cerezyme® infusions every 4 weeks versus every 2 weeks in the maintenance therapy of patients with type 1 Gaucher disease**
Atul Mehta, Royal Free Hospital and University College of London, London

11.45–12.00 **Joint discussion**

12.00–12.25 **Recent advances in type 3 Gaucher disease**
Ashok Vellodi, Great Ormond Street Hospital for Children, London

12.25–12.55 **Existing and novel ERT approaches**
Ari Zimran, Shaare Zedek Medical Centre, Jerusalem

12.55–14.00 *Restaurant Floor 0*

LUNCH BREAK

8TH EUROPEAN WORKING GROUP ON GAUCHER DISEASE (EWGGD) MEETING
JUNE 5, 2008

Thursday

14.00–16.00 *Star Auditorium Floor -1*

SESSION 3
Other therapies

Chairs: *Derralynn Hughes,* Royal Free & University College Medical School, London
Carla Hollak, University of Amsterdam, Amsterdam

SRT

14.00–14.15 **Long-term results of substrate reduction therapy for Gaucher disease**
Deborah Elstein, Shaare Zedek Medical Centre, Jerusalem

14.15–14.30 **Experience with SRT**
Pilar Giraldo, University of Zaragoza, Zaragoza

14.30–14.45 **Bone mineral density and SRT**
Gregory Pastores, New York University School of Medicine, NY

14.45–15.00 **Joint discussion**

Novel therapy approaches

15.00–15.15 **Preliminary results of a phase 2 clinical trial of Genz-112638 in patients with type 1 Gaucher disease**
Elena A. Lukina, National Research Centre for Haematology, Moscow

15.15–15.30 **A phase 2 clinical trial of the pharmacologigal chaperone AT2101 for the treatment of Gaucher disease**
Neil J. Weinreb, University Gaucher Treatment Center, Tamarac, FL

15.30–15.40 **Joint discussion**

15.40–16.00 **Development of a novel orally administered non-viral gene therapy for Gaucher disease using DNA nanoplexes encapsulated in yeast cell wall particles**
Edward Ginns, University of Massachusetts Medical School, Worcester, MA

16.00–16.20 *Room Jasmine Floor 0*

TEA BREAK

16.20–17.40 *Star Auditorium Floor -1*

SESSION 4
Neuropathology

Chairs: *Miguel Pocovi,* University of Zaragoza, Zaragoza
Bruno Bembi, Centre for Rare Disorders, Udine

16.20–16.35 **Neurological complications in type 1 Gaucher disease**
Carla Hollak, University of Amsterdam, Amsterdam

16.35–16.45 **The neurological manifestations of type 1 Gaucher disease: The French Observatoire on Gaucher Disease (FROG)**
Patrick Cherin, University Pierre et Marie Curie Paris VI, Paris

16.45–16.55 **Parkinsonism in relatives of Gaucher patients heterozygous for GBA1-mutations**
Ralf Hartung, University Children's Hospital, Mainz

16.55–17.10 **Joint discussion**

17.10–17.40 **Development of novel therapies in murine models for Gaucher disease**
Stefan Karlsson, Lund University Hospital, Lund

17.40–18.00 *Room Jasmine Floor 0*

TEA BREAK

18.00–19.00 *Star Auditorium Floor -1*

INVITED LECTURE 2

Chair: *Timothy Cox,* University of Cambridge, Cambridge

Rare diseases: a European perspective
György Kosztolányi, University of Pécs, Pécs

19.30 *Hotel Lobby Floor 0*

FREE EVENING

08.30–19.00 *Registration Desk Floor 0*

REGISTRATION

09.00–10.40 *Star Auditorium Floor -1*

SESSION 5
Science and industry

Chairs: *Hans Aerts,* University of Amsterdam, Amsterdam
Atul Mehta, Royal Free Hospital and University College of London, London

09.00–09.40 ***Genzyme***
From a small miracle to a small molecule and beyond: Genzyme's commitment to the Gaucher community
Richard Moscicki, Genzyme Corporation, Cambridge, MA

09.40–10.05 ***Shire Human Genetic Therapies***
Differential in vitro responses in inflammatory and immune cytokine production elicited by enzyme replacement therapies for Gaucher disease
Paolo Martini, Shire HGT, Boston, MA
Andrew Onderdonk, Harvard Medical School, Boston, MA

10.05–10.25 ***Protalix Biotherapeutics***
Novel enzyme replacement therapy for Gaucher disease: On-going phase III clinical trial with recombinant human glucocerebrosidase expressed in plant cells
Einat Almon, Protalix Biotherapeutics, Carmiel

10.25–10.40 ***Amicus Therapeutics***
Scientific rationale for the use of pharmacological chaperone in Gaucher disease
Brandon Wustman, Amicus Therapeutics, Cranbury, NJ

10.40–11.00 *Room Jasmine Floor 0*
COFFEE BREAK

11.00–12.30 *Star Auditorium Floor -1*
EUROPEAN GAUCHER ALLIANCE

Launch of the Susan Lewis Memorial Fund & patient led initiatives
Members of the European Gaucher Alliance (EGA)

12.30–13.30 *Restaurant Floor 0*
LUNCH BREAK

13.30–15.00 *Star Auditorium Floor -1*
SESSION 7
Debates on future directions

13.30–14.00 **Debate 1: Unmet clinical need**
Chairs: *Bruno Bembi,* Centre for Rare Disorders, Udine
Timothy Cox, University of Cambridge, Cambridge

14.00–14.30 **Debate 2: Unresolved science questions**
Chairs: *Helen Michelakakis,* Institute of Child Health, Athens
Hans Aerts, University of Amsterdam, Amsterdam

14.30–15.00 **Debate 3: Remaining diagnostic challenges**
Chairs: *Maria Clara Sa Miranda,* IBMC Institute for Molecular and Cell Biology, Porto
Ben Poorthuis, University of Amsterdam, Amsterdam

15.00–15.30 *Room Jasmine Floor 0*
TEA BREAK

8TH EUROPEAN WORKING GROUP ON GAUCHER DISEASE (EWGGD) MEETING
JUNE 6, 2008

Friday

15.30–17.00 *Star Auditorium Floor -1*
PLENARY DISCUSSION ON FUTURE DIRECTIONS

Chairs: *Bruno Bembi*
Timothy Cox
Helen Michelakakis
Hans Aerts
Maria Clara Sa Miranda
Ben Poorthuis

17.00–17.20 *Star Auditorium Floor -1*
DISCUSSION ON PREGNANCY ISSUES

Chair: *Melinda Erdős,* University of Debrecen, Debrecen
Judit Tóth, University of Debrecen, Debrecen

17.15–17.25 **Pregnancy and delivery in females with Gaucher disease**
Eugen Mengel, University Children's Hospital, Mainz

17.25–17.35 **Management of reproductive events in females with Gaucher disease**
Ari Zimran, Shaare Zedek Medical Centre, Jerusalem

17.20–18.00 *Room Jasmine Floor 0*
TEA BREAK

18.00–19.00 *Star Auditorium Floor -1*

INVITED LECTURE 3

Chair: *Hans Aerts,* University of Amsterdam, Amsterdam

Recent advances in gene therapy of LSDs with CNS manifestations
Timothy Cox, University of Cambridge, Cambridge

19.30 *Meeting Point in Hotel Lobby Floor 0*

DINNER ON EUROPA BOAT

08.30–13.00 *Registration Desk Floor 0*

REGISTRATION

09.00–10.40 *Star Auditorium Floor -1*

SESSION 8
Advances in the laboratory

Chairs: *Martin Hrebicek,* Charles University, Prague
Mia Horowitz, Tel Aviv University, Tel Aviv

09.00–09.20 **Biomarkers of avascular necrosis in Gaucher disease**
Elena Pavlova, University of Cambridge, Cambridge

09.20–09.35 **Chitotriosidase-monitoring during pregnancy in women with Gaucher disease**
Verena Lehmann, University Children's Hospital, Mainz

09.35–09.55 **Yet another glucocerebrosidase?**
Hans Aerts, University of Amsterdam, Amsterdam

09.55–10.15 **ER associated degradation and unfolded protein response in Gaucher disease patients**
Mia Horowitz, Tel Aviv University, Tel Aviv

10.15–10.25 **A nonsense mutation in the LIMP-2 gene associated with progressive myoclonic epilepsy and nephrotic syndrome**
Maria Clara Sá Miranda, Porto University, Porto

10.25–10.40 **Joint discussion**

10.40–11.00 *Room Jasmine Floor 0*

COFFEE BREAK

11.00–12.50
Star Auditorium
Floor -1

SESSION 9
Poster session and oral presentation of selected abstracts

Chairs: *Gregory Pastores,* New York University School of Medicine, New York, NY
Helen Michelakakis, Institute of Child Health, Athens

11.00–11.30 **Poster viewing**

11.30–11.40 **Plasmalogen levels in Gaucher disease**
Helen Michelakakis, Institute of Child Health, Athens

11.40–11.50 **Common variants in the glucosylceramide synthase gene are associated with disease severity in Gaucher disease patients**
Pilar Alfonso, University of Zaragoza, Zaragoza

11.50–12.00 **Bone marrow transplantation for acute myeloid leukemia from donor with Gaucher disease followed by Enzyme Replacement Therapy (ERT)**
Mirando Mrsic, University Hospital Zagreb, Zagreb

12.00–12.10 **Validation of saccadic latency as a biomarker of cerebral injury in lysosomal storage disorders**
Jonathan Roos, University of Cambridge, Cambridge

12.10–12.20 **Chaperone effect of several iminosugars and aminocyclitols on mutated glucocerebrosidases as a possible therapeutic approach for Gaucher disease**
Lluïsa Vilageliu Arqués, University of Barcelona, Barcelona

12.20–12.30 **Inefficacy of drilling in early stages of osteonecrosis in Gaucher disease**
Ehud Lebel, Shaare Zedek Medical Centre, Jerusalem

12.30–12.40 **Immune thrombocytopenia in type I Gaucher disease**
Hanna Rosenbaum, Rambam Medical Centre, Haifa

12.40–12.50 **Gaucher disease: a model to study the role of glycosphingolipids in atherosclerosis**
Johanna Groener, University of Amsterdam, Amsterdam

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12.50–13.00
Star Auditorium
Floor -1

POSTER PRIZES
ANNOUNCEMENT OF NEXT MEETING
CLOSING OF THE MEETING

Chair: *Pramod Mistry,* Yale University School of Medicine, New Haven, CT
Hans Aerts, University of Amsterdam, Amsterdam

13.00–14.00
Restaurant
Floor 0

LUNCH

Saturday

Invited papers

Yet another glucocerebrosidase?

Aerts J.M., Dekker N., Donker W.E., Verhoek M., Hollak C.E., Strijland A., Boot R.G.
Department of Medical Biochemistry, Academic Medical Center, University of Amsterdam

Gaucher disease is remarkably heterogeneous in clinical manifestation, even among individuals with a similar glucocerebrosidase genotype. This heterogeneity has stimulated interest in the existence of other cellular glucosidases that might compensate the deficiency of lysosomal glucocerebrosidase (GBA1), and thus effect clinical manifestation .
In this presentation, the features of GBA2, originally coined non-lysosomal glucosylceramidase, are firstly discussed. GBA2 is clearly capable of hydrolyzing glucosylceramide, but its cellular localization is outside the lysosome. No indications have been found by us for genetic heterogeneity in GBA2 effecting clinical outcome in GBA1-deficient GD patients.
Next, attention is focussed to another cellular beta-glucosidase, GBA3, originally named non-specific or broad-specificity beta-glucosidase. The existence of this enzyme has been recently re-discovered by Ito and coworkers and they unfortunately re-named it Klotho-related protein (KLrP). In contrast to findings of several earlier investigations, Ito and coworkers have proposed that GBA3/KLrP is a true glucocerebrosidase and that heterogeneity in the enzyme might underlie phenotypic variation among GD patients with identical GBA1 genotype. This view sharply contrast with the earlier belief that GBA3 is playing a role in catabolism of xenobiotic beta-glucosides rather than glucosylceramide. To shed light on the topic, we have re-examined the features of GBA3/KLrP and studied its relationship with GD manifestation. The preliminary outcome of this investigation will be discussed.

Recent advances in gene therapy of LSDs with CNS manifestations

Cox T.M.
Department of Medicine, University of Cambridge, UK.

The lysosomal diseases in general, and Gaucher disease in particular, occupy a special position in the rapid translation of discoveries in molecular cell biology to clinical practice. Characterization of these single-gene disorders has contributed much to the amelioration of their somatic manifestations by enzyme therapy using recombinant glycoproteins preferentially targeted to the diseased organelle. At the same time, competitive commercial interest has invested in alternative agents, including substrate inhibitors and pharmaceutical chaperones, which have avanced rapidly to late-phase clinical development. However the principal biochemical classes of lysosomal disorder affect the brain and lead to severe white matter disease or relentless neuronal injury. Oral drugs, based on small molecules with the potential to penetrate brain tissue, are superficially attractive for these manifestations - but it remains uncertain as to whether any agent that does not fully restore the underlying enzymatic defect will ever improve the neurological outcome. On the other hand, gene therapy has the potential definitively to correct deficiency of soluble lysosomal proteins and, like enzyme replacement therapy, takes advantage of unique secretion-recapture pathways to restore lysosomal defects by functional complementation.
Latterly there have been striking successes in the development of vectors for the safe transfer of therapeutic genes of interest to non-mitotic cells in which they can be expressed in the long term. These are principally lentiviral and adeno-associated viral vector systems; vectors based on the herpes simplex virus, which is trophic for neurons, have also attracted attention. At the same time, the burgeoning availability of spontaneous and genetically manipulated animals with lysosomal defects that faithfully recapitulate cognate human disorders, permits authentic experimentation to be conducted as a prelude to clinical

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studies in humans. Successful and safe use of recombinant adeno-associated viral vectors recently in human Parkinson's disease inspires immense optimism for lysosomal disorders affecting the brain; and in several neurodegenerative lysosomal diseases there have been spectacular successes in advanced experimental models.

Here I will present successful applications of gene therapy in the different classes of lysosomal disorders in which neurodegeneration is prominent. Safe application of gene therapy has long been awaited in clinical medicine; but these fields of research are at last opening up for credible discovery in the lysosomal diseases – and rich rewards, with deserved gratitude, are promised for those who dare to invest in them.

Long-term results of substrate reduction therapy for Gaucher disease

Elstein D.
Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel

Prior to 1990 and the advent of enzyme replacement therapy (ERT), therapeutic options for symptomatic Gaucher disease were limited to splenectomy (total or partial), orthopedic surgeries, ancillary treatments, and hopes raised by potentially curative modalities such as organ and bone marrow transplants, and gene therapy. None of the latter three have proven sufficiently effective to halt disease progression without considerable risk. Although ERT with imiglucerase (Cerezyme, Genzyme Therapeutics, Cambridge MA) with an excellent safety profile has revolutionized care for the visceral features, the collective experience has been that "enzyme therapy is not enough" (G. Grabowski, 2001). There is a need for modalities that can effectively impact brain, bone, and lung as well as reduce/eliminate dependency on intravenous infusions. Oral substrate reduction therapy (SRT) with the iminosugar miglustat (Zavesca, Actelion Pharmaceuticals, Allschwil Switzerland) has been tested in several clinical trials and has been approved for marketing in several countries because of benefits that have been reported. The effect on organ reduction and hypersplenism is acceptable, albeit with a longer trajectory than with Cerezyme, and efficacy is in direct correlation with compliance. A tangential study of cognitive function in type 1 patients resulted in the first hint that miglustat traverses the blood-brain barrier and possibly effects visual-spatial functioning, a parietal lobe function known to be affected in Gaucher disease. There have also been studies of the effect of miglustat on bone and anecdotal experiences of the effect in patients with echocardiographic evidence of pulmonary hypertension. Nonetheless, the adverse events profile is still worrisome: although the GI side effects can be controlled, the peripheral neuropathy has not been adequately explained and some patients, despite withdrawal of drug, continue to suffer from neurological symptoms, thus raising concerns about long-term exposure to the drug.

Monitoring Skeletal Response in Type 1 Gaucher Disease

Goldblatt J.
School of Paediatrics and Child Health, University of Western Australia

Skeletal complications in Type 1 Gaucher disease likely result from a combination of pressure effects of the accumulating storage cells and altered bone modelling homeostasis, as a consequence of "effector leakage" from damaged monoctye/macrophage cells. The extent of skeletal involvement is not always related to other evidence of overall disease burden and suggests local factors are involved in determining the severity of bone damage. Irreversible bone complications, generally a result of avascular necrosis, clearly do not respond to therapies such as enzyme replacement. During enzyme replacement therapy it is therefore critical to monitor the skeletal response to enable titration of dose to the lowest level without compro-

mising bone integrity. The comprehensive Australian National Gaucher Treatment Program essentially monitors skeletal disease by centralised assessment of annual skeletal MRI's done according to a set protocol, with severity scored on a semi-quantitative bone marrow burden (BMB) scale. Adjunctive evidence of response is derived from associated testing of the biomarker chitotriosidase and bone mineral density measures. The MRI derived BMB monitoring has been found to provide the best marker of bone response and improved scores have correlated with reduction in symptoms and the lack of development of irreversible complications.

Neurological complications in type 1 Gaucher disease

Hollak C.E.M., Biegstraaten M., Aerts J.M.F.G., Van Schaik I.N.
Department of Medical Biochemistry, Academic Medical Center, University of Amsterdam

Gaucher disease is classically divided into three phenotypes. Type 1 Gaucher disease is traditionally differentiated from type 2 and 3 disease by the absence of nervous system involvement. However, an increasing number of reports has emerged on neurological manifestations in patients with type 1 Gaucher disease. Whether a strict division in three different phenotypes is still valid has been the subject of debate. We reviewed the available literature on this subject and identified eighty-five studies in which type 1 Gaucher disease patients or carriers of a glucocerebrosidase mutation were described with a neurological disease or a condition which is known to be associated with neurological disease. In addition, we investigated retrospectively a large Dutch cohort of type 1 Gaucher disease patients for the prevalence of neurological manifestations. At the same time, we are involved in a large prospective study on the prevalence and incidence of polyneuropathies and other co-morbidities in type 1 Gaucher disease as part of a multinational study under the auspices of the European Working Group on Gaucher Disease.
The literature search revealed that central nervous system disease in type 1 Gaucher disease could be roughly classified into four groups: misclassification of patients that had attenuated onset of typical type 3 manifestations, Parkinsonian manifestations, myelum compression as a result of skeletal disease or malignancy and a miscellaneous group of sporadic disorders. Peripheral nervous system disease was reported as peripheral neuropathy (mono- and polyneuropathies, including small fiber neuropathy in one study), dorsal root compression as a consequence of skeletal disease and cranial nerve palsies. The nature of the neurological disease manifestations in type 1 Gaucher disease was confirmed in our Dutch cohort (n=75 patients): a diagnosis of a neurological disease was made 34 times in 75 patients during a median follow-up time of eleven years, including Parkinson disease, type 3 misclassification, dementia, peripheral neuropathies and facial nerve palsies. In addition, the baseline data from the prospective cohort study (n=103 patients) showed that 11 (10.7%) patients were diagnosed with sensory or sensory / motor axonal polyneuropathy (95%CI, 5.5–18.3%). This prevalence is significantly higher than in the general population (0.12–3.6%). Patients with polyneuropathy were older than those without polyneuropathy (mean [SD] age, 61.1 [10.3] vs. 40.4 [13.4] years, respectively), and had a higher mean (95%CI) level of plasma chitotriosidase (13856.9 [5202.4–22511.2] vs. 7236.9 [5566.5–8907.3] nmol/ml.h, respectively).
In conclusion, the term non-neuronopathic Gaucher disease does not seem to be an appropriate characterization of type 1 Gaucher disease. However, the neurological signs and symptoms in type 1 Gaucher disease are of a total different kind and, in the majority of cases, of much less severity in comparison with the signs and symptoms associated with type 2 and 3 disease Therefore, type 1 disease should be classified as a separate phenotype.

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ER associated degradation and unfolded protein response in gaucher disease patients

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In Gaucher disease mutations in the gene encoding lysosomal glucocerebrosidase lead to accumulation of glucosylceramide(s) mainly in cells of the reticuloendothelial system. Therefore, two abnormal conditions should be considered in this disease, namely, presence of mutant proteins and accumulation of substrates. Both, may have deleterious effects. We focused on the fate of mutant glucocerebrosidase variants. We showed that one of the factors that determine Gaucher disease severity is the ER associated degradation (ERAD) process of mutant glucocerebrosidase. In this process misfolded or unassembled proteins are recognized by the ER quality control machinery, eliminated from the ER to the cytosol by a retrograde transport and finally eliminated by the ubiquitin-proteasome system. Our results also showed that ERAD of mutant glucocerebrosidase variants leads to ER stress, which triggers the unfolded protein response (UPR) and activation of transcription of two proteins: the Bip (Grp78) chaperone and the transcription factor CHOP (GADD153), which are known components of the UPR. ER stress is affected by intracellular free cholesterol. We therefore tested the effect of cholesterol on mutant glucocerebrosidase variants. Our results showed that growing Gaucher cells that originated from patients with a phenotype more severe than that predicted from their mutations in cholesterol depleted medium led to lessening in the degree of ERAD and thus to improvement in stabilization, maturation, lysosomal localization and activity of the mutant glucocerebrosidase variants. The same effect was achieved by treating the cells with the HMG CoA reductase inhibitor, mevastatin. Non of the treatments had a significant effect on glucocerebrosidase properties in normal cells or in cells that derived from mildly affected patients, indicating that intracellular cholesterol is one of the factors that affect the ERAD process of glucocerebrosidase and may influence the severity of Gaucher disease. Treatment of normal cells or cells that originated from mild Gaucher disease patients with U18666A, a drug that disturbs cholesterol trafficking, increased the levels of ER retained glucocerebrosidase and lowered its lysosomal fraction, indicating that cholesterol homeostasis is an important factor in trafficking of glucocerebrosidase.

Gaucher Disease and Cancer

Hughes D.
Royal Free & University College Medical School, London

Gaucher Disease, the most common of the lysosomal storage disorders, results from deficiency of β-glucocerebrosidase.[1] Consequent accumulation of glucosylceramide in reticuloendothelial cells leads to characteristic macrophage storage cells and multi-organ pathology.[2] In addition to the direct effects of cellular storage, such as hepato-splenomegaly and bone marrow infiltration, a number of defects of humoral immunity have been described. Cytokines including IL-6 and M-CSF have been found to be elevated[3;4] and several studies have detected increased levels of polyclonal and monoclonal immunoglobulins.[5-9] Separate studies in both Israel and Europe have described an apparent predisposition to malignancy in small cohorts of patients with Gaucher disease[10;11] and suggested that haematological malignancy occurs at a rate 12-15 fold higher than in the general population. However, a larger study of 2742 patients from the Gaucher registry recorded no higher overall risk of malignancy but confirmed a six fold higher rate of one type of haematological malignancy, multiple myeloma.[12] Little is understood regarding the role of glucosyl ceramide accumulation in immune system pathology or in the development of multiple myeloma in Gaucher Disease. It has been suggested that lipid storage results in chronic stimulation of the immune system[13] possible by reducing the size of the Natural Killer T cell population.[14] It is unclear whether polyclonal elevation of immunoglobulins, monoclonal gammopathy and multiple myeloma in Gaucher disease represent a spectrum of pathologies with a common aetiology or whether there is progression between the entities in an individual patient.

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Development of Novel Therapies in Murine Models for Gaucher Disease

Enquist I.B., Nilsson E., Lo Bianco C., Ooka A., Månsson J.E., Ehinger M., Brady R.O., Richter J., Karlsson S.
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Gaucher disease (GD) is an autosomal recessive lysosomal storage disorder, caused by mutations in the glucosidase, beta, acid (GBA) gene that encodes the lysosomal enzyme glucosylceramidase (GCase). GCase deficiency leads to characteristic visceral pathology and in some patients, lethal neurological manifestations. Previous mouse models with GCase deficiency have either been lethal in the perinatal period or viable without displaying clinical features of GD. We have generated viable mice with characteristic clinical symptoms of type 1 GD (hepatosplenomegaly, anemia, etc.), by conditionally deleting GCase exons 9-11 upon induction postnatally. Both transplantation of wild type (wt) bone marrow (BM) and gene therapy through retroviral transduction of BM from GD mice prevented development of disease as well as corrected an already established GD phenotype. The gene therapy approach generated considerably higher GCase activity than transplantation of wt BM. Strikingly, both therapeutic modalities normalized glucosylceramide levels and practically no infiltration of Gaucher cells could be observed in BM, spleen and liver demonstrating correction at 5-6 months following treatment. The findings demonstrate for the first time the feasibility of gene therapy for type 1 GD in vivo. Using similar approaches, we have generated mouse models for severe neuronopathic Gaucher disease. To circumvent the lethal skin phenotype observed in several of the previous GCase-deficient animals, we genetically engineered a mouse model with strong reduction in GCase activity in all tissues except the skin. These mice exhibit rapid motor dysfunction associated with severe neurodegeneration and apoptotic cell death within the brain, reminiscent of

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neuronopathic GD. In addition, we have created a distinct mouse model, in which GCase deficiency is restricted to neural- and glial cell progenitors and progeny. These mice develop similar pathology as the first mouse model, but with a delayed onset and slower disease progression, indicating that GCase deficiency within microglial cells which are of hematopoietic origin is not the primary determinant of the CNS pathology. These findings strongly suggest that normal microglial cells cannot rescue this serious neurodegenerative disease. In summary, our mouse models can be used to investigate pathological mechanisms and develop novel therapies for all types of Gaucher Disease.

Insulin resistance in Gaucher disease

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Type II diabetes and Gaucher disease, seemingly unrelated conditions that differ strongly in their etiology, may have a surprising similarity in their pathophysiology, the common denominator being disturbances in glycosphingolipid metabolism.
In obesity induced insulin resistance, a condition preceding type II diabetes, increased glycosphingolipid levels are the result of increased substrate availability. Elevated palmitate concentrations, the hallmark of obesity induced insulin resistance, lead to increased glycosphingolipid synthesis. Complex glycosphingolipids, especially GM3 are present in the cell membrane in close proximity to the insulin receptor. GM3 may interfere with insulin action, most likely at the level of autophosphorylation of the insulin receptor, resulting in a decrease in insulin stimulated glucose uptake. Evidence for the interference of GM3 with insulin receptor signaling comes from in vitro and animal studies. GM3 synthase knockout mice display higher insulin sensitivity and are protected from high fat diet induced insulin resistance. In 3T3-L1 adipocytes, TNFα-induced insulin resistance is accompanied by an increase in GM3, resulting in impaired insulin signaling. Depletion of GM3 restores insulin signaling in these cells. In mouse models of obesity induced insulin resistance pharmacological inhibition of conversion of ceramide to glycosphingolipids improves insulin sensitivity.
In Gaucher disease, GM3 concentrations in plasma and several cell types are elevated. We performed a hyperinsulinemic euglycemic clamp, which indicated that Gaucher disease is associated with peripheral insulin resistance. In a second study we addressed the question whether this disease related insulin resistance, possibly in combination with weight gain during enzyme replacement therapy (ERT), would result in an increased incidence of type II diabetes. In untreated Gaucher patients the prevalence of overweight is lower than in the general population. Long term treatment with ERT induces a larger than average weight gain leading to a similar prevalence of overweight in enzyme therapy treated patients and the general population. The prevalence of type II diabetes increases significantly during treatment with ERT, resulting in a comparable prevalence of type II diabetes in enzyme therapy treated patients and the general population.
In conclusion, Gaucher disease is associated with decreased insulin sensitivity, presumably caused by elevated GM3 levels. Treatment with ERT or substrate reduction results in partial correction of the elevated ganglioside concentrations, which may enhance insulin sensitivity. On the other hand, treatment induced obesity may negatively influence insulin sensitivity. The net result is as yet unknown, but we hypothesize that patients remain at increased risk for the development of type II diabetes and should therefore be closely monitored.

A multicenter, randomized, dose frequency study of the safety and efficacy of Cerezyme® infusions every 4 weeks versus every 2 weeks in the maintenance herapy of patients with type 1 Gaucher disease

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Introduction: Cerezyme(R) (imiglucerase, Genzyme Corporation) is the current standard treatment for type 1 Gaucher disease (GD1). Most patients are infused every 2 weeks. A less frequent infusion schedule at the same total 4-week dose might be equally effective and more convenient. *Objective:* To compare the safety and efficacy of two dosing frequencies of Cerezyme for improved convenience for patients with GD1. *Methods:* A phase IV, multicenter, randomized 24-month trial enrolled clinically stable adult GD1 patients who had received Cerezyme years. Patients were randomized to continue their total 4 week dose as 1 infusion every 2 weeks (Q2) or 1 infusion every 4 weeks (Q4). The primary analysis used a composite end-point of relative change in hemoglobin level, platelet count, liver and spleen volumes, or progression of bone disease and bone crisis. A post-hoc analysis was performed based upon maintenance of therapeutic goals. *Results:* Mean 4-week doses (U/kg) of Cerezyme were 70.4±24.9 for Q2 (n=33) and 69.7±21.3 for Q4 (n=62). The primary analysis endpoint was maintained in 80.8% of Q2 and 63.2% of Q4 patients at 24 months. Per the post-hoc analysis, 100% of Q2 and 88.5% of Q4 patients maintained therapeutic goals at 24 months. No Cerezyme-related serious adverse events were reported. *Conclusions:* Infusing a total 4-week dose of imiglucerase every 4 weeks appears to be safe and well-tolerated in the majority of patients and may be considered for some stable adult GD1 patients who have achieved therapeutic goals on Cerezyme therapy.

SRT effects on bone involvement in Gaucher disease

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Proof of principle for substrate reduction therapy (SRT) in Gaucher disease (GD) using miglustat received regulatory approval both within the EU countries and the United Sates, based on demonstration of its relative safety and efficacy in stabilizing and\or improving the hematologic and visceral manifestations. As bone involvement can represent a source of significant morbidity, related to bone pain, 'crises', pathologic fracture and osteonecrosis with arthropathy, it is important to establish that the longterm use of miglustat can lead to control or improvement in the bone manifestations as well. As the pattern of bone involvement in GD extends to both the trabecular and cortical compartments, monitoring must incorporate assessments of both to ascertain the rate and magnitude of change. Limited data on quantitative chemical shift imaging has shown changes in signal intensity that likely correspond to hematopoietic reconstitution within the lumbar vertebra. Data on the use of DEXA indicates a small, but statistically significant improvement in bone mineral density in both the lumbar spine and femoral neck. These findings, which necessitate confirmation in a larger cohort, provide a rationale for the use of SRT in GD. Thus far, there are no safety concerns that would restrict the use of SRT, and the introduction of miglustat provides both patients and practitioners the option to consider an oral agent in the management of GD. As GD is a chronic disorder requiring lifelong therapy, it will be critical to ascertain the longterm safety and efficacy of miglustat in patients switched from enzyme therapy. It will also be of interest to evaluate the the of bisphosphonates in combination, or serially among patients receiving miglustat. Issues that will require resolution include determination of the patients most suitable to receive SRT, and the selection of the most appropriate option with increase in the number of therapeutic choices.

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LIMP-2 Is a Receptor for Lysosomal Mannose-6-Phosphate-Independent Targeting of ß-Glucocerebrosidase

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A variety of acid hydrolases mediate the degradation of different substrates within the lysosome. Most lysosomal enzymes are targeted in a mannose-6-phosphate receptor dependent pathway to the lysosome. In contrast, the acid β-glucocerebrosidase (βGC), the enzyme defective in Gaucher disease, is directed by a so far unknown mechanism to the lysosome - independently of the mannose-6-phosphate receptor.
Affinity-chromatography experiments revealed that the lysosomal integral membrane protein LIMP-2 is a specific binding partner of βGC. This interaction involves a coiled-coil domain within the lumenal domain of LIMP-2. βGC activity and protein levels were severely decreased in LIMP-2-deficient mouse tissues. Analysis of fibroblasts and macrophages isolated from these mice indicated that the majority of βGC was secreted. Missorting of βGC was also evident in vivo, as protein and activity levels were significantly higher in sera from LIMP-2-deficient mice compared to wild-type. Reconstitution of LIMP-2 in LIMP-2-deficient fibroblasts led to a rescue of βGC levels and distribution. LIMP-2 expression also led to lysosomal transport of a βGC endoplasmic reticulum retention mutant. Our data support a role for LIMP-2 as the mannose-6-phosphate-independent trafficking receptor for βGC. The presentation will summarize our recent insight into the cellular trafficking of βGC and the newly identified interaction with LIMP-2.

Reczek, D.; Schwake, M., Schröder, J., Hughes, H., Blanz, J., Jin, X., Brondyk, W., VanPatten, S., Edmunds, T., Saftig, P. (2007) LIMP-2 Is a Receptor for Lysosomal Mannose-6-Phosphate-Independent Targeting of ß-Glucocerebrosidase. Cell 131; 770-783.

Saposin C deficiency

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Gaucher disease is generally caused by a deficiency of the lysosomal enzyme glucocerebrosidase, resulting from mutations in the glucocerebrosidase GBA gene.
In addition to this specific hydrolase, the degradation of glycosphingolipids requires the participation of small glycoprotein called sphingolipid activator protein C (SAP C).
The prosaposin PSAP gene codes for a single protein which undergoes post-translational cleavage to yield four proteins named saposins A, B, C and D. Saposin SAP C is required for glucosylceramide degradation and its deficiency results in a variant form of Gaucher disease. Three of described up to 2007 patients with SAP C deficiecy displayed neuronopathic form of Gaucher disease. These cases had a similar mutational pattern, with a missense mutation in the saposin C domain on one allele (p.C382F, p.C382G, p.C315S), and another mutation abolishing the production of all saposins (unknown, p.Q430X, p.M1V) on the other allele. Last year the first case of SAP C deficiency was described in non-neuronopathic form of Gaucher disease in two siblings. These patients were identified thanks to the high level of chitotriosidase as a marker which was pivotal for further investigations; and it led to the proper diagnosis.A molecular genetics study of the PSAP gene in these patients with non-neuronopathic form identified two mutations in the heterozygous state: one missense mutation, p.L349P, located in the SAP-C domain, and another mutation, p.M1L, located in the initiation codon of the prosaposin precursor protein gene. Probably such genotype ensure a minimal activity of glucocerebrosidase sufficient for glucocerebroside degradation in central nervous system.

Recent advances in type 3 Gaucher disease

Vellodi A.
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Two topics wil be discussed. The first is neural plasticity and how this might be altered in NGD. The second is the role of the exocytic machinery that delivers perforins and granzymes, the means by which cytotoxic T cells and NK cells kill their targets, in the pathogenesis of NGD.

Dose-response relationships in Gaucher disease

Vom Dahl S.
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Background: Dosing of enzyme replacement therapy (ERT) for Gaucher disease type 1 varies from 15 to 130 U/kg/month, making a huge economic difference. The most effective dose in the initial years of treatment is not known. Further, knowledge about the needed dose during the different phases of treatment is incomplete. *Aims:* To examine dose-response issues in Gaucher type I treatment with enzyme replacement therapy, either alglucerase or imiglucerase in the first 2-10 years of treatment. *Methods:* Retrospective analyses from the International Collaborative Gaucher Group (ICGG)-based Gaucher Registry (n= 366 and n=191), a retrospective analysis in a two-cohort study of Dutch-German patients (n=106) and a prospective multi-center study on bone mineral density (BMD) in a cohort of 342 GD type I patients. *Results:* Different recent approaches, including registry analyses, either propensity scoring-based or cumulative (3,4), a retrospective analysis in a two-cohort Dutch-German study (1) and a prospective analysis in a multi-center trial (2) show quite consistent results. There is a dose-response relationship for the following parameters: increase of hemoglobin (1,4), increase of platelets (1,4), decrease of liver size (1,4), decrease of spleen size (1,4), chitotriosidase (1), improvement of bone marrow burden score (1) and increase of bone mineral density (2). Further, on average, patients who received higher doses of imiglucerase achieve a greater number of defined therapeutic goals (5) within a given time period (3). *Conclusions:* Although some analyses have clearly shown that the therapeutic outcomes of enzyme supplementation therapy are dose-dependent for the induction and stabilization phase of enzyme replacement therapy, e.g. the first 3-10 years, the differences for hematological and visceral parameters are marginal, whereas bone-related responses (bone marrow burden, bone mineral density) are better and easier to reach with higher doses of imiglucerase/alglucerase. The cumulative number of therapeutical goals to be reached within a given time period is associated with the given dose. Based on these studies, the definition of realistic quantitative therapeutical goals must therefore precede the assignment of enzyme dose in Gaucher disease (6). Normalization and improvement of bone manifestations may take many years. Little is known about tapering and maintenance in ERT. There is lack of comparative studies on clinical endpoints like death, morbidity, hospitalizations, quality of life or major bone complications.

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Free papers selected for oral presentation in the meeting programme

The neurological manifestations of Gaucher's disease type 1: The French Observatoire on Gaucher Disease (FROG)

Cherin P.[1], Rose C.[2], De Roux-Serratrice C.[3], Tardy D.[4], Dobbelaere D.[5], Grosbois B.[6], Hachulla E.[7], Jaussaud R.[8], Javier R.M.[9], Noel E.[10], Clerson P.[11], Hartmann A.[12]

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Introduction: Type 1 Gaucher disease (GD1), the most common variant, is classically considered non-neuronopathic. This French national prospective study was implemented for describing the clinical aspects of an adult GD cohort. The patients were systematically evaluated for the presence of neurological signs and symptoms. *Patinent and methods:* Clinical data with complete neurological assessment were collected during a routine visit. No additional tests were performed. The CRF was designed for guidance of the physician as an educational tool. *Results:* From May 2005 to September 2006, 105 type 1 GD patients were included (mean age 45.6+/-13.7 (m+/-SD) years. Thirty-five percents of patients had at least one sibling with GD and 19% had a family history of Parkinson's disease. Out of the thirty-eight patients with genotyping, 71% had a N370S mutation and 29% had at least one L444P or D409H mutation. Fifty-one patients presented with at least one neurological symptom. Three patients (aged 63, 67 and 70 years old) suffered from Parkinson's disease and 23 had Parkinson symptoms. In comparison with the patients without Parkinson's signs, these 23 patients were older (53+/-14 vs 43+/-12 years, p = 0.001) and diagnosis of GD was performed later (31+/-16 vs 24+/-12, p=0.03). Myoclonia occured in 6 patients, peripheral neuropathies in 5 and epilepsy in 2 patients. *Conclusion:* These data challenge the current classification suggesting a continuum between neurologic and non-neurologic forms in Gaucher's disease. Evaluation of therapeutic options for the neurological dysfunction of GD1 is also needed.

A new type I Gaucher disease severity score index for phenotypic classification and evaluation of responses to treatment

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Background: Gaucher disease is the first lysosomal storage disease for which specific therapy became available. Over 4800 patients have been treated with enzyme replacement therapy. Analysis of International Gaucher Registry data has outlined the clinical heterogeneity and the different responses to treatment from patient to patient, and for different organs. This heterogeneity justifies the development of a severity score index to assess disease activity, stage and prognosis, and to quantify the effects of treatment. *Design/method:* The new scoring system , the "Gaucher Disease Severity Score Index - Type I" (GauSSI-I), is based on the clinical experience of the authors and on an extensive literature review. In particular for skeletal disease, all the available scoring systems have been reviewed and compared in order to provide a skeletal scoring that allows the use of any of the different methods on an equivalent basis. An adjusted Delphi technique was used to reach a consensus on the severity score. *Results:* The new scoring system, GauSSI-I, has been developed. Six specific domains, in which different items were scored according to their impact on morbidity, have been characterised. GauSSI-I was validated in 53 type I Gaucher patients treated with imiglucerase, and it was compared to the Zimran score, the only severity index score available so far. *Conclusion:* The GauSSI-I is a reliable method for staging the severity of adult type I Gaucher disease, and it is more sensitive than the Zimran score for monitoring the response to treatment

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Development of a Novel Orally Administered Non-Viral Gene Therapy for Gaucher disease using DNA Nanoplexes Encapsulated in Yeast Cell Wall Particles

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An ingestable formulation of hollow, porous yeast cell wall particles (YCWP) encapsulating DNA nanoplexes encoding therapeutic proteins is being developed as a non-viral gene therapy for Gaucher disease. Following oral administration, the YCWP-DNA payload is internalized by intestinal M cells, released into underlying tissue and rapidly and efficiently taken up by macrophages. As macrophages migrate to tissues, DNA is released from the acidifying endosome into the cytoplasm, and processed by cellular machinery to active therapeutic proteins such as human glucocerebrosidase, huGBA. YCWP-huGBA DNA formulations have been used to introduce huGBA into murine macrophages in culture, as well as to treat long-lived Gaucher mice generated by gene targeting. Murine macrophages treated in-vitro with YCWP-huGBA DNA efficiently expressed huGBA. Administration of YCWP-GBA DNA formulations to Gaucher mice resulted in extensive particle uptake. Compared to untreated Gaucher mice, oral administration of YCWP-GBA DNA formulations significantly increased liver GBA activity and decreased tissue Gaucher cells in treated mice. Results of a small pilot study also suggest that this therapy sufficiently corrects tissue GBA activity to ameliorate symptoms in treated, compared to untreated, severely affected Gaucher mice. The ability of this ingestible macrophage targeted gene therapy to improve delivery and restore huGBA activity to tissues suggests that this approach could achieve significant reversal of tissue pathology, including bone. In addition to enabling a safer, more efficient and cost effective treatment for Gaucher disease, this macrophage targeted therapeutic strategy could be used to treat a wide range of other medical conditions, including low bone density and inflammatory diseases.

Parkinsonism in relatives of Gaucher patients heterozygous for GBA1-mutations

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Introduction: In the literature an association between Gaucher disease and Parkinsonism has been shown. Due to these findings a study in Israel found an increased number of heterozygote carriers for Gaucher disease in Parkinsonism patients. However this study was limited by the high frequency of only a few Gaucher mutations in this specific population. *Patients and methods:* 74 of non-neuronopathic Gaucher patients were interviewed or asked by a standardized questionnaire to identify relatives with signs of Parkinsonism. All Gaucher patients and relatives with Parkinsonism, confirmed by a neurologist, were genotyped. Additionally the degree of relationship, the age and date of onset as well as the symptoms were

noted. *Results:* From 74 patients out of 65 families, 56 out of 47 families provided efficient informations of their relatives. In this 47 families 11 relatives with Parkinsonism were detected. The mean age at diagnosis was 51,5 years. The relationship to the Gaucher patients were: 3 x parent, 4 x grandparent, 1 x great-grandparent and 3 x sibling of a parent. Only one of the 8 relatives with a 50 % chance of carrier status did not carry a Gaucher allele. The age range of Gaucher patients was between 4 and 70 years (mean: 32,5 years). 56 % of the Gaucher alleles carried the mutation N409S, 13 % L483P, 4 % RecNcil, 3 % del55. All other mutations were rare mutations. In 6 % of the alles no mutation was found. The prevalence in the Mainz cohort of the common mutations is comparable to the number found in the Gaucher registry. *Conclusions:* These results seem to confirm the higher prevalence of Parkinsonism in a representative population group of heterozygote carriers of Gaucher disease.

Chitotriosidase-Monitoring during pregnancy in women with Gaucher disease

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Chitotriosidase is a widely used biomarker, which reflects total storage burden in Gaucher patients. Marked decrease of chitotriosidase activity is observed in patients after initiation of ERT. The aim of our study was to monitor chitotriosidase activity during pregancy in women with Gaucher disease. *Patients and results:* In 5 pregancies in 5 women with Gaucher diease we were able to monitor chitotriosidase. 3 had ongoing ERT without changing the dosage regime during pregnancy. 1 moderate affected woman was observed without ERT before and during pregancy. The 5th woman has interrupted ERT after conception. ERT was restarted after amelioration of Gaucher disease symptoms in the 26th week of gestation. In the 3 woman with ongoing ERT as well as in the woman without ERT chitotriosidase activity decreased during pregnancy. After delivery the acivity increased to levels higher than before pregnancy. These four woman reported well-being during the pregnancy and extreme fatigue in the weeks after delivery. Interruption of ERT in the 5th woman was followed by worsening of thrombopenia and rapid increasing spleen volume. Parallel chitotriosidase was doubled. Reinstition of ERT caused an rapid decline of chitotriosidase activity and an increase of thrombocyte count. Delivery was without complication. *Discussion:* Spontanously decreasing chitotrisidase activity during pregnancy goes hand in hand with subjective self-reported well-beeing. Worsening of Gaucher disease was reflected biochemically by increasing chitotriosidase activity. We conclude that monitoring with chitotriosidase during pregnancy may documents worsening or amelioration of Gaucher disease. Physiological immunological and hormonell changes during pregancy may have a positive effect regarding Gaucher disease.

Preliminary Results of a Phase 2 Clinical Trial of Genz-112638 in Patients with Type 1 Gaucher Disease

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Introduction: Genz-112638 is a novel oral small molecule inhibitor of glucosylceramide synthase for the treatment of Gaucher disease type 1 (GD1). *Objective:* To assess the efficacy, safety, and pharmacokinetics

of Genz-112638 in patients with GD1. *Methods:* An ongoing open-label Phase 2 clinical trial of Genz-112638 (50 or 100mg bid orally) enrolled patients with GD1 in Israel, North America, Russia, and South America. The main efficacy endpoints of the study were changes in haemoglobin level, platelet count, and spleen volume after 52 weeks. An extension study will follow. Results: To date, 26 weeks of follow-up data were available for 8 patients receiving Genz-112638. All 5 males and 3 females (age range: 18-33y) were Caucasian 1 was of Jewish descent. The mean change in hemoglobin was 1.3±0.5g/dL and the mean percentage change in platelet count was 42.7±20.7%, with all patients showing increases following 6 months of therapy. Mean percentage changes for spleen and liver volume were -29.9±8% and -10.1±6.4%, respectively all patients showed reductions in organ size following 6 months of therapy. Plasma glucosylceramide levels normalized in all 8 patients. Mean percent change in chitotriosidase level was -42.5%. One drug-related adverse event was reported for these 8 patients that was mild and transient in nature. *Conclusions:* Initial observations suggest that Genz-112638 may represent a safe, effective, and convenient oral therapy for patients with GD1. Based upon the clinical results obtained thus far, clinical development of Genz-112638 will proceed in ongoing and additional clinical trials.

Pregnancy and delivery in females with Gaucher disease

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In the past only few publications, which described mainly the jewish patient-cohort, addressed pregnancy and delivery complications in females with Gaucher disease. Our cross-sectional study focused retrospectively on the natural history of pregnancy and delivery in females with Gaucher disease in the pre-ERT-aera and documented prospectively the same issues in females with ERT. Patients and results: In this study 50 women older than 21 years had been included. 25 woman had at least one successful pregnancy. In summary they have born 37 children, 9 with ERT in pregnancy and 28 without ERT. Additional 2 abortions (maternal reasons) and 6 stillborns were noted. Major Gaucher disease complications were not observed during pregnancy. Only 5 babies (4 without / 1 with ERT) were preterm. Mean birth weight 3173g +/- 513 SD differs not from the birth weight in the German normal population. A significant number of postpartal bleeding events were noted. 7/28 women needed transfusion after delivery due to postpartal bleeding in the pre-ERT-erea, whereas 1/9 woman had a blood transfusion with ERT in pregnancy ($p<0,01$). ERT-infusion related reactions were not observed during pregnancy. Discussion: ERT during pregnancy in women in our European cohort with moderate to severe Gaucher disease type 1 is safe. Moreover in our study the frequency of peripartum bleeding associated with Gaucher disease is significant lower in the group of women, who had ERT during pregnancy. Our findings encourage to continue imiglucerase treatment throughout pregnancy.

Biomarkers of Avascular Necrosis in Gaucher Disease

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To investigate whether chemokines and cytokines are related to bone manifestations of Gaucher disease, we conducted multiplex assays in 100 adults with clinically categorized disease as part of the UK bone research consortium.
Mean age was 45 years (18-86); 92 were receiving imiglucerase (median duration 8 years; (2-18). Forty

three had experienced avascular necrosis (AVN); and eight failed therapeutic goals, with AVN despite enzyme therapy. Eighteen serum cytokines/chemokines were determined by fluorimetric bead arrays in Gaucher patients and healthy volunteers (10 male and 10 female). Intra-assay variations were 2-9.8%; inter-assay variation was 5.6-15%.
IL-1β, IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-α, IL-5, FGF basic, IL-1α were undetectable. VEGF and RANTES did not differ between Gaucher and control samples. Concentrations of MIP-1α, MIP-1β, MCP-1, IL-8, IL-1ra and CCL18/PARC were elevated in Gaucher patients ($p<0.05$ for each). MIP-1β, IL-8 and CCL18/PARC concentrations were greater in the 43 AVN patients (MIP-1β median 88.6 pg/mL; IL-8 median 30.5 pg/mL; CCL18/PARC median 434 ng/mL) compared with the 57 AVN-free patients (medians of 59.4, 13.3 and 283, respectively, $p<0.05$). Moreover, the eight patients failing the therapeutic goal had concentrations of MIP-1α, MIP-1β, IL-8 and CCL18/PARC (medians 73.2; 120.9; 36.3 pg/mL and 767ng/mL), which significantly exceeded the values in 84 patients who had met this goal (medians 52.3; 71.2; 16.5 pg/mL and 315ng/mL, respectively, $p<0.05$). Treatment exposures were similar.
Conclusion: Numerous serum cytokines are elevated in Gaucher disease. CCL18/PARC, MIP-1α, MIP-1β and IL-8 are biomarkers associated with avascular necrosis- and may allow this disabling complication to be predicted.

A nonsense mutation in the LIMP-2 gene associated with progressive myoclonic epilepsy and nephrotic syndrome

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Recently it has been described by Reczeck et al. (Cell, 2007 131,770-783) that LIMP-2 is the trafficking receptor for b-glucocerebrosidase. Here we present a Portuguese family with two siblings showing a progressive myoclonic epilepsy without intellectual impairment and a nephrotic syndrome. The biochemical analysis revealed a severe deficiency of b-glucocerebrosidase activity in cultured skin fibroblasts but a normal enzymatic activity in leukocytes. The patient did not present activated macrophages and increased levels of chitotriosidase, the hallmark of Gaucher disease. These findings suggested a defect in the intracellular pathway of b-glucocerebrosidase. Molecular studies of the gene encoding LIMP-2 confirmed this hypothesis. A homozygous nonsense mutation in codon 178 of LIMP-2 gene was found in the patient whereas healthy parents were heterozygous for the mutation. The western blot analysis of patient fibroblasts confirmed the absence of LIMP-2 and revealed a decrease and an abnormal glycosylation pattern of b-glucocerebrosidase. The small amount of b-glucocerebrosidase detected in these cells was Endo-H sensitive suggesting a localization at the endoplasmic reticulum. The study also proved that b-glucocerebrosidase was mainly located in endoplasmic reticulum, as assessed by its sensibility to Endo H. Altogether these findings confirm the role of LIMP-2 as the mannose-6-phosphate independent sorting receptor for b-glucocerebrosidase in human fibroblasts but also suggest that in other cells, namely in macrophages, alternative mechanisms must be responsible for targeting b-glucocerebrosidase to the lysosomes.

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Development of a Disease Severity Scoring System for Type 1 Gaucher Disease

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Introduction: A validated disease severity scoring system (DS3) for type 1 Gaucher disease (GD1) is needed to monitor progression and treatment response in individuals and to compare patient cohorts in clinical studies. *Objective:* To develop and test the reliability and validity of a DS3 to assess and monitor GD1 in routine clinical care and to stratify for disease severity in clinical studies. *Methods:* DS3 domains were established by an expert physician group using nominal group technique (NGT) consensus formation methodology. Items within domains were selected by Delphi survey of 32 international physician experts in GD1. The expert group analyzed survey data (including preliminary screening for reliability, feasibility, and face, content, discriminant, and predictive validity) to determine appropriate measurement techniques for each variable. Measurements were weighted considering how much morbidity and mortality each contributes to GD1. Severity scores for sample patient cases determined by NGT were compared to scores determined by the DS3 and the Zimran SSI, an existing severity scoring index for GD1. *Results:* The DS3 includes bone, hematological, visceral, and physician- and patient-reported domains. Clinical domains were populated with weighted measurements of GD1 signs and symptoms. Patient case scores assigned by the expert group were more highly correlated with DS3 scores (Pearson r=0.98) than those obtained with the existing SSI index (Pearson r=0.88). *Conclusions:* This provisional DS3 provides for accurate assessment of GD1 status. Testing of reliability and validity will continue for ultimate implementation of the DS3 in clinical practice and trials.

A phase 2 clinical trial of the pharmacological chaperone AT2101 for the treatment of gaucher disease

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AT2101 (isofagomine tartrate) is an oral pharmacological chaperone that selectively binds to wild type and mutant ß-glucocerebrosidases (GCase) in the endoplasmic reticulum, increases enzyme stability, trafficking to the lysosome, and cellular activity. A randomized, open-label clinical trial of AT2101 in imiglucerase-treated patients with Type 1 Gaucher disease (GD1) was conducted at 11 U.S. sites. The primary objective was to evaluate safety and tolerability of different doses and dosing regimens. The secondary objective was to evaluate GCase and other biomarkers of GD1. Imiglucerase infusions were suspended for 2 weeks washout, 4 weeks of AT2101 dosing, and one week follow-up. Thirty adult subjects were enrolled 29 completed the study and 1 subject withdrew from the study after 2 doses of AT2101 due to an unrelated adverse event of moderate bone pain. AT2101 was generally well tolerated at all doses and schedules evaluated. There were no serious adverse events. The majority of adverse events were graded mild or moderate and not related to the study drug. GCase activity as measured in leukocyte lysates was increased in 20 of 26 evaluable patients. The magnitude of GCase increase differed across dose groups and genotypes. Hematologic and other laboratory measures, including biomarkers of GD, remained stable. Longer term trials to evaluate clinical efficacy and safety of AT2101 for GD1 will soon begin.

Management of reproductive events in females with gaucher disease

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Background: Disease-specific treatment for type 1 Gaucher disease has been available since 1991 in the form of alglucerase (enzyme replacement therapy) and later its recombinant form, imiglucerase. Treatment goals and monitoring guidelines for imiglucerase therapy are established (Pastores et al, 2004 Weinreb et al, 2004) but do not make specific reference to females for whom Gaucher disease could have impact during reproductive events. *Aims:* To examine the reciprocal effects of reproductive events and Gaucher disease in untreated and enzyme-treated females and to identify optimal management. *Methods:* International clinicians experienced in Gaucher disease management convened to review evidence from the peer-reviewed literature, an alglucerase and imiglucerase pharmacovigilance database, and their own clinical experience to support recommendations. *Results:* Menarche may be delayed in untreated symptomatic girls. Menorrhagia seems more common than in non-Gaucher females. Women may be at increased risk of complications during pregnancy and delivery because of peripartum bleeding and worsening bone involvement. Alglucerase or imiglucerase therapy before and during pregnancy may help reduce spontaneous abortions and risk of peripartum complications. There is no evidence of teratogenicity associated with alglucerase and imiglucerase, or any adverse effect on breast fed infants. Breast feeding may present a physiological challenge to mothers with Gaucher disease. *Conclusions:* Menarche and pregnancy are the main issues. Planned conception, and a multidisciplinary approach to pregnancy management, is the most desirable route to parenthood. Women treated with imiglucerase prior to pregnancy should be allowed (or encouraged) to continue imiglucerase treatment throughout pregnancy and breast feeding (despite the package insert warning). *References:* Pastores GM, Weinreb NJ, Aerts H, et al (2004) Therapeutic goals in the treatment of Gaucher disease. Semin Hematol41(4 Suppl 5):4-14. Weinreb NJ, Aggio MC, Andersson HC et al (2004) International Collaborative Gaucher Group (ICGG). Gaucher disease type 1: revised recommendations on evaluations and monitoring for adult patients. Semin Hematol.41(4 Suppl 5):15-22.

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Free papers selected for oral presentation at the poster session

Common variants in the glucosylceramide synthase gene are associated with disease severity in gaucher disease patients

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Gaucher disease (GD), caused by a deficiency of the glucocerebrosidase (GC) enzyme, is characterized by the accumulation of glucosylceramide (GlcCer) within lysosomes, resulting in cellular dysfunction and damage. GlcCer is catalyzed by the glucosylceramide synthase (GCS), also known as UDP-glucose ceramide glucosyltransferase, (UGCG), EC 2.4.1.80, first enzyme in glycosphingolipids biosynthesis. Mutations in the glucocerebrosidase (GBA) gene are required to cause GD, but other factors play an important role. GCS gene is a logical candidate to hypothesize that its variability could be involved in clinical severity. We have analyzed GCS variants (g.(-295)C>T, g.(-222)ins10, g.148A>G, g.166A>T, g.25510C>G, g.29312A>G and g.34991A>G) in a group of 80 [N370S]+[L444P] heterozygous and 23 N370S homozygous patients. Results were related with severity score index (SSI). g.(-295)C>T and g.166A>T SNPs were in complete linkage disequilibrium. In the N370S homozygous, carriers of g.(-222)ins10 have higher SSI than non carriers (6.6 vs 2.4, $p<0.004$). Patient carriers of the A-allele for SNP g.148A>G had higher SSI than the G-allele carriers (6.7 vs 1.5, $p<0.001$, for [N370S]+[L444P] heterozygous and 4.3 vs 1.7, $p<0.001$, for [N370S] homozygous). "In silico" studies indicate that 10bp insertion at the promoter region generates a new ETF binding site and the g.148A>G variant at intron 1 change the affinity for the transcription factor AP-2. In conclusion, our results suggest that the genetic GCS variants could influence in the development and progression of GD severity.

Gaucher disease: A model to study the role of glycosphingolipids in atherosclerosis

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Objective: Gaucher diseases is a sphingolipidosis caused by a deficiency of lysosomal glucocerebrosidase leading to accumulation of glucosylceramide, mainly in macrophages. Plasma of these patients contains low levels of HDL-cholesterol, high levels of apoE and increased levels of glucosylceramide and ganglioside GM3. In this study we have further characterized the individual lipoproteins in plasma of Gaucher patients in view of their possible atherogenicity. *Methods:* Fasted plasma from three untreated Gaucher patients and three age and gender matched controls was used. Very low density (VLDL), low density (LDL), and high density lipoproteins (HDL) were isolated by Redgrave gradient ultracentrifigation and the lipid composition, including ceramide and glycospingolipids, of VLDL, LDL, and HDL was estimated. Nuclear magnetic resonance (NMR) spectroscopy was used to determine the subclass concentration and mean sizes of VLDL, LDL, and HDL in the three untreated Gaucher patients. *Results:* Gaucher patients had significantly lower HDL cholesterol levels, increased levels of triglycerides and increased levels of apoE. Glucosylceramide (GlcCer) was 3-fold increased (Gaucher plasma 17.1 ± 3.8 nmol/ml control plasma 5.7 ± 1.5 nmol/ml). Ceramide (Cer) levels were lower in plasma from Gaucher patients (Gaucher plasma 6.9 ± 1.1 nmol/ml control plasma 9.0 ± 1.8 nmol/ml). Based on the NMR spectroscopy measurements Gaucher patients had a higher risk

subclass profile consisting of small size HDL and a larger number of small sized LDL particles. The increased level of GlcCer was reflected in all lipoproteins. The GlcCer/Cer ratio was lower in VLDL (Gaucher 0.77 vs. control 0.19) compared to LDL (Gaucher 2.73 vs. control 0.60), and HDL (Gaucher 4.84 vs. control 1.28) in Gaucher patients as well as controls. *Discussion:* We showed that in addition to low HDL-cholesterol levels Gaucher patients have small sized HDL particles and increased number of small sized LDL particles, a profile that is associated with high risk for atherosclerosis. Despite this atherogenic profile Gaucher patients appear not at increased risk for atherosclerosis (1). This strongly indicates a role for glycosphingolipids in lipoprotein metabolism and development of atherosclerosis and makes Gaucher disease an excellent human model to study this. The difference in GlcCer/Cer ratio in the individual lipoproteins as found in Gaucher disease patients and controls point to a different origin of these glycosphingolipids in plasma lipoproteins. It is hypothesized that in human plasma GlcCer found in lipoproteins is derived at least in part from macrophages probably via HDL, and ceramide is derived mainly from liver as part of newly synthesized VLDL. 1. See abstract M. Langveld et al at this EWGGD meeting.

Inefficacy of drilling in early stages of osteonecrosis in Gaucher disease

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Background: Gaucher disease is characterized by clinical heterogeneity. One of the most devastating consequences of the disease is bone involvement, affecting a majority of patients and often despite specific enzyme treatment. Clinically, skeletal disease ranges in severity from mild osteopenia to osteonecrosis of joints in very young patients, but no markers have been discovered to predict onset and/or progression. Drilling (or "core decompression") of osteonecrotic bone has been advocated for pre-collapse stages of femoral head osteonecrosis (ARCO 1-2). The current study describes the experience of our large referral center using drilling for joint osteonecrosis in relatively young patients with Gaucher disease. *Methods:* We retrospectively reviewed medical data of all patients recommended to undergo small diameter drilling for osteonecrosis of juxta-articular bone of the femoral head, humeral head or upper tibia for acute osteonecrosis in a pre-collapse stage, ARCO stages 1-2. *Results:* 11 patients (mean age = 34years) underwent drilling of 12 bones with juxta-articular necrosis 3 additional patients (mean age = 51years) refused intervention. Nine joints that underwent drilling showed advancing joint degeneration within 0.5 to 4 years, three have undergone replacement of the three joints that did not undergo drilling, two have undergone replacement and one has is collapsed with osteoarthritis. *Conclusions:* We conclude that conservative measures such as preserving range of joint motion, aspiration of joint effusion, narcotic analgesics, and psychological support will involve fewer side effects with the same short-term benefits as drilling in short, we can not provide evidence of superior results due to drilling in patients with Gaucher disease. It is clear that only by improving our understanding of bone physiology and patho-physiology will we be able to intervene effectively.

Plasmalogen levels in Gaucher disease

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Plasmalogens represent a unique type of phospholipids characterized by the presence of a vinyl-ether bond at the sn-1 position of the glycerol backbone. Peroxisomes are essential in their biosynthesis. Their suggested functions include protection against oxidative stress, participation in signal transduction, membrane

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fusion events, cholesterol transport and membrane trafficking, processes known to be disturbed in sphingolipidoses. We here report on red blood cell membrane (RBC) plasmalogen levels in Gaucher disease (GD) patients. Plasmalogen levels were measured as their dimethylacetal derivatives (DMA) by gas chromatography in lipid exctracts of erythrocytes from 15 patients. Their relative amount was estimated as the ratio between C18:0 DMA and methylstearate (C18:0), as well as C16:0 DMA and methylpalmitate (C16:0). Statistically significant lower levels of both plasmalogen species were observed in GD patients compared to normal individuals. Furthermore, a negative correlation between plasmalogen levels and chitotriosidase was observed in the patients, which was statistically significant for the C18:0 species. Upon therapy a significant rise of plasmalogen levels and fall in chitotriosidase activity was observed. However, C18:0 DMA/C18:0 was still significantly lower in GD patients compared to controls and the negative correlation to chitotriosidase persisted. At both time points there was no indication of an overt peroxisomal dysfunction, very long chain fatty acid, phytanate and pristanate levels being normal. In conclusion, reduced plasmalogen levels that show a significant rise following treatment and a negative correlation to total disease burden, as expressed by chitotriosidase activity, are observed in GD.

Bone marrow transplantation for acure myeloid leukemia from donor with gaucher disease followed by enzyme replacement therapy (ERT)

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Gaucher disease is the most common lysosomal storage disorder. The disease is extremely serious, disabling and can be mortal in the less or distant future without treatment. Enzyme Replacement Therapy (ERT) with imiglucerase prevents progressive manifestations of the disease. Allogeneic hematopoietic stem cell transplantation (HSCT) is performed for the treatment of leukemia, aplastic anemia and severe combined immunodeficiency. Before the era of ERT, HSCT despite limited availability (HLA identical donor) has been used for the treatment of Gaucher disease. Case report. A 32 year old female without any history of underlying disease was admitted to the hospital due to high number of leukocytes. Diagnosis of standard risk acute myeloid leukemia was established in May 2002. Disease remission after one standard chemotherapy cycle was obtained in June 2002. HLA test were performed and HLA identical brother was found. He had a long history of anemia bone pain. Splenectomy was done in his childhood. According to the medical documentation Gaucher disease was mentioned but never diagnosed. Because availability of ERT and severe underlying disease (acute leukemia) we decided to proceed with HSCT despite donor underlying disease (Mb. Gaucher). Donor examination prior to donation was as follows: hemoglobin level 110 g/L, platelet 148 x109/L, WBC in normal range. Bone MR was revealed typical changes for Gaucher disease. Beta glucosidase level was low (2.4 nmol/h/mg of protein) and chitotriosidase very high (60 000 nmol/h/ml of plasma). There were no other signs of other diseases and we decided to proceed with HSCT. Patient were conditioned with standard busulfan plus cyclophosphamide regimen followed by graft versus host disease (GVHD) prevention with combination of cyclosporine and short course of methotrexate. Marrow harvesting was complicated due to the lacking to collect sufficient number of cells and two leukapheresis was performed. After hematopoietic stem cell infusion recovery was successful and patient was discharged form the hospital on day +35. There were no signs of acute or chronic GVHD. Complete chimerism was obtained from marrow and blood. There were no signs of Gaucher disease in bone marrow. Donor ERT was started immediately after hematopoietic stem cell donation. Recipient ERT was started 3 months after hematopoietic stem cell infusion. There were no signs of Gaucher disease in the recipient. Due to administrative reasons ERT was stopped after 2 years of treatment. Recent examinations showed that the recipient started to develop signs of Gaucher disease (bone changes, bone pain and elevation of chitotriosidase). ERT was introduced again in the recipient and maintained now for 6 years. Recent examination showed no signs of underlying disease

as well as reduction signs of Gaucher disease. *Conclusion:* This case report shows that hematopoietic stem cells of donor with Gaucher disease can be successfully transplanted. Harvesting could be complicated due to the insufficient number of collected cells. Enzyme Replacement Therapy (ERT) can prevent the development of Gaucher disease in the recipient transplanted with Gaucher marrow.

Validation of saccadic latency as a biomarker of cerebral injury in lysosomal storage disorders

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Objective: We sought to validate the use of saccadic latencies – the delay before an eye moves in response to a target – as a measure of cerebral injury in patients with lysosomal storage diseases. *Methods:* We used a simple infra-red oculometric device with head-mounted laser targets to determine saccadic latencies in five patients with Sandhoff disease and one with Type III Gaucher disease. *Results:* Saccadic reaction times were greatly prolonged in the five Sandhoff patients when compared with 56 healthy control subjects (mean 441msec compared with 167msec, $p<0.00001$). Latency correlated with clinical scoring systems such as the gross motor function test ($R2=0.952$) as well as with the results of diffusion tensor imaging. On repeat examination lateral variation in patients was not significant ($p>0.14$) and results were independent of background luminance. Unexpectedly, the more usual saccadic parameters, such as duration, amplitude, peak velocity, and hence by implication HSEM-alpha, did not discriminate between Sandhoff patients and control subjects ($p >0.09$, 0.43, 0.09 t-test). The type III Gaucher patient, who was only subtly impaired, had latencies at the higher reference range. *Conclusions:* The European Medicines Agency has questioned the use of several saccadic eye movement parameters in therapeutic trials. Our results suggest that, unlike such brainstem-derived responses, median saccadic latency represents a robust and sensitive method to quantify cognitive function at the bedside. Saccadic latency may be usefully applied to test innovative treatments for neuronopathic storage disease.

Immune Thrombocytopenia in Type I Gaucher Disease

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Backgound: Type I Gaucher disease (GD) the non-neuronopathic form is characterized by hepatosplenomegaly, pancytopenia and skeletal complications due to the accumulation of glucocerebroside in macrophages. Thrombocytopenia is usually related to hypersplenism and/or infiltration of bone marrow by the lipid-laden macrophages namely Gaucher cells. Enzyme replacement therapy (ERT) restores the hemoglobin and platelet count in treated GD patients within 12-24 months of treatment. In GD patients, including ERT treated, with persistent low platelet counts other ethiological factors should be considered. Goals: To determine the etiology of persistent thrombocytopenia in Type I GD patients and to evaluate their clinical course and hematological parameters. Methods: Flow cytometric technique was used to detect platelet-surface associated IgG/M (PSIgG/M) in a cohort of 24 Type I GD patients followed at the Gaucher clinic in Haifa, Israel. The evaluated hematological parameters of the thrombocytopenic GD patients include: bleeding phenomena, concurrence of autoimmune phenomena, hematological malignancies and bone marrow findings. Results: Twenty four Type I GD patients, 15 females and 9 males with an age range of 35 to 80 years (median 53 years) were included in the study. Seventeen of the evaluated 24 patients were thrombocytopenic with platelet counts less than <100x109/l and 7 /24 were in the normal range. Bone marrow aspirate was performed in 16 of the 17 thrombocytopenic patients and showed normal or hyperplasic megakariopoiesis together with Gaucher cells infiltrates. Six of the 17 thrombocytopenic patients received ERT for at least 24 months with no effect on the low platelet counts. Elevated platelet surface IgG was detected in 16/17(94%) of GD patients with thrombocytopenia and in only 1/7 (14%) of non thrombocytopenic patients ($p<0.0001$). In 6/17 of the thrombocytopenic patients, surface IgM (PSIgM) was found, in addition to the PSIgG. Those six patients are known with monoclonal IgM (concomitant Waldenstrom macroglobulinemia), markedly elevated polyclonal IgM levels, or lupus like autoimmune disorder which may have been responsible for the positive PSIgM. Only three thrombocytopenic patients with platelet counts less than 40x109/l had bleeding tendency (mainly purpura) with no response to steroid treatment (two of them were also resistant to ERT concerning their thrombocytopenia). *Conclusions:* Thrombocytopenia in Type I GD is related to either infiltration of bone marrow compromising megakariopoiesis or hypersplenism, but immune factors should also be considered. Despite the lack of response to steroids, the observed megakaryocytic hyperplasia in Gaucher infiltrated marrows and the presence of platelet surface antibodies in the thrombocytopenic patients, strongly implicate autoimmune etiology. In persistent thrombocytopenic patients immune factors should be defined. The present study demonstrates that surface platelet antibodies may play a role in refractory thrombocytopenic GD patients. Since the role of splenectomy is controversial in GD, immune modulation approach should be considered.

Chaperone effect of several iminosugars and aminocyclitols on mutated glucocerebrosidases as a possible therapeutic approach for Gaucher disease

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Gaucher disease is a glycosphingolipid disorder, caused by deficiency of lysosomal acid ²-glucosidase (GBA) due mainly to mutations on the GBA gene. It results in progressive accumulation of glucosylceramide in lysosomes of macrophages of reticuloendothelial system. Some competitive inhibitors, at subinhibitory concentrations, can work as chemical chaperones, increasing the activity of wild-type and mutated GBAs. We have tested the effect of two iminosugars, N-(n-nonyl)-deoxynojirimycin (NN-DNJ) and N-(n-butyl)-deoxynojirimycin (NB-DNJ), and four aminocyclitols (C4-Ph, C8, C9 and C10) on COS-7 cells transfected with N188S, G202R, E326K, N370S, G377S, I402T, D409H, L444P, N188SE326K, and H255QD409H and on patient fibroblasts. NN-DNJ, when tested at different concentrations in stable cell lines, lead to a 1,2-1,4 fold increase in the activity of wild-type, N188S and G377S GBAs and a slight increase in the activity of the N188SE326K GBA. For the other iminosugar, NB-DNJ, also an increase in activity (1,2 fold at 5 microM) was observed in cells transfected with N188S and N188SE326K cDNAs. The aminocyclitol C4-Ph showed a remarkable effect on the activity of, again, N188S, and N188SE326K GBAs, as well as on wild-type enzyme. No significant effects were observed for the other mutated enzymes and/or for the compounds C8, C9 and C10. In fibroblasts, positive results were obtained for the treatment with NN-DNJ on cells bearing genotypes D409H/N188SE326K, N370S/N370S and N370S/L444P Also, for treatment with the aminocyclitol C10 on L444P/G202R and in L444PE326K/G202R patient cells. The positive but somehow limited results presented here, encourage the search for new products to be tested as chaperones.

Free papers selected for poster presentation

Identification and functional characterization of four novel mutant alleles causing Gaucher disease

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Gaucher disease (GD) is the most prevalent lysosomal storage disorder. It is caused by the defective activity of the acid lysosomal β-glucosidase (GBA) resulting in the accumulation of sphingolipids in the macrophages of different tissues. Clinically, patients with GD have been classified as type 1 (GD1) non-neuronopathic, type 2 (GD2) neuronopathic, and type 3 (GD3) chronic neuronopathic phenotypes. The GBA gene is located on chromosome 1 and contains 11 exons. A highly homologous pseudogene is located 16 kb downstream from the active gene.
We report here the identification of 4 novel GBA mutant alleles, including three missense mutations, P245T, W381C and N188I, and one complex allele, N188S+G265R.
Mutations P245T, W381C were found in two GD1 patients, as compound heterozygote with N370S, the most common mutation in GD1 patients. The mutant allele N188I was found in a GD2 patient in association with mutation R131C, while the complex allele N188S+G265R was present in a GD3 patient who was heterozygous for this allele and an uncharacterized mutation.
The mutant proteins were expressed in vitro in COS-1 cells and assayed for the enzymatic activity and protein processing. The N370S allele was used as a control and expressed an average activity of 25 % with respect to the wild type activity. All mutants proteins were inactive
To further characterized the N188S+G265R allele, we also expressed in vitro the constructs bearing the single mutations (N188S and G265R). While G265R mutant expressed no activity, the I188S expressed an activity of 50% with respect to the wild type activity, as previously reported. It is interesting to note that the enzymatic activity of the two proteins bearing mutations at position N188 (N188S and N188I) is completely different. The change of Asn, with Ser (both polar aminoacids) leads to the synthesis of an enzyme that retains a quite high residual activity, while the change of Asn with Ile (a non polar aminoacid) completely abolish the enzymatic activity.
Western blot analysis showed that cells transfected with constructs bearing mutations P245T, W381C and N188I, expressed reduced levels of protein. Cells transfected with the G265R mutant construct did not express GBA protein, while the double mutant expressed fairly amount of protein, suggesting that the presence of I188S probably prevent the degradation of the G265R protein.

Conflicting results in leucocytes and fibroblasts in two unusual cases of gaucher disease

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Gaucher disease is caused by a reduction or total deficiency of glucocerebrosidase, and characterised by gross elevation of plasma chitotriosidase activity. This study presents 2 cases where deficiency of glucocerebrosidase was not expressed in all tissues investigated.
Case 1: The index case showed a severe hydrops presentation. Prenatal diagnoses were performed on cultured amniocytes in two at risk fetuses, showing glucocerebrosidase deficiency on both occasions (2.0 and 2.3 nmol/hr/mg; control range 25-138, mean = 71.6, n = 18). A 3rd prenatal diagnosis was performed directly on uncultured villus; showing reduced but not deficient activity considered to be compatible with a

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carrier fetus (10.3 nmol/hr/mg, control 21). A severely hydropic baby was born at 29 weeks gestation. Leucocyte glucocerebrosidase activity was normal (1.1 nmol/hr/mg, NR 1-5), however chitotriosidase activity was markedly elevated (318 nmol/hr /ml; NR 4-120). The index case showed a C16S homozygous genotype, which was also confirmed in this baby.
Case 2 presented at age 3 years. Initial investigation found leucocyte glucocerebrosidase activity to be normal (1 nmol/hr/mg; NR 1-5) and plasma chitotriosidase to be deficient. Further investigation again showed normal glucocerebrosidase activity (1.9 nmol/hr/mg; NR 1-5) and chitotriosidase deficiency. Subsequently glucocerebrosidase was found to be reduced in cultured skin fibroblasts (10.4 nmol/hr/mg, assay controls 71, 240) confirming the diagnosis of Gaucher disease.
Glucocerebrosidase activity towards artificial substrates may be expressed differently in various tissues. Normal activity in leucocytes may not exclude a diagnosis of Gaucher disease; it may be necessary to also perform the assay on cultured cells.

Serum chitotriosidase activity in heterozygotes for chitotriosidase deficiency (24 bp duplication) Gaucher patients on enzyme replacement therapy

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Serum chitotriosidase activity (SCT) is known to be many times increased in Gaucher patients. During the enzyme replacement therapy (ERT) it decreases being a good marker of therapy efficacy. Quite significant number of individuals present a deficiency of SCT activity due to 24 bp duplication in exon 10 in chitotriosidase gene. In the presented study, SCT in Gaucher patients heterozygous for chitotriosidase deficiency and those with wild genotype was compared. In the examined group of 72 unrelated Gaucher patients we found 2 cases (i.e.2.9 %) with SCT deficiency (SCT <2 nmoles/ml/hr). The incidence of SCT deficiency among 421 unrelated patients suspected of a lysosomal disease was similar (13 of 421 patients i.e. 3.1%). The DNA analysis for 24 bp duplication performed in a group of 64 patients (128 alleles) revealed 17 mutated alleles (13%). In Gaucher patients heterozygous for 24 bp duplication SCT was about twice times lower than in wild genotype and this difference is statistically significant (median values 6315 and 14362 nmoles/ml/hr, n=13 and 39, respectively, $p < 0.001$). After 3 to 5 years of ERT, SCT decreased in both groups - namely in heterozygotes for chitotriosidase deficiency the median value dropped to 901 nmoles/ml/hr, and in wild genotype to 1290 nmoles/ml/hr. The cessation of Ceredaseâ/Cerezymeâ administration caused a similar increase of SCT in both groups. Our data show that SCT is a good marker of treatment conditions also in heterozygotes for chitotriosidase deficiency allele.

Response to enzyme replacement therapy with velaglucerase alfa, gene-activated human glucocerebrosidase, in a patient with type 1 gaucher disease (GD)

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Aim: To evaluate the response in a patient treated with an investigational human enzyme replacement therapy, velaglucerase alfa. *Background/methods:* Enzyme replacement therapy is considered the gold standard for the treatment of type 1 GD. velaglucerase alfa is a novel ERT in development for the treatment of patients with type 1 GD. This glycoprotein is produced from a well-characterized continuous human cell line using Shire HGT proprietary gene-activation technology and has an identical amino acid sequence to

the naturally occurring human enzyme. A 26-year old female of Spanish/South American Indian descent, has been found to have massive hepatosplenomegaly, anemia, retarded growth, progressive fatigue, and malaise. From January 1999 to February 2001 she received alglucerase (Ceredase®) at a dose of 32U/kg every other week. Response was good, but treatment was discontinued because of unavailability of the enzyme in Paraguay. Considerable progression of the disease was noted thereafter in subsequent clinical follow up. Starting in October 2005, she began receiving 60 U/kg of velaglucerase alfa every other week. The patient had been on 24 months of velaglucerase alfa treatment at 60U/kg at the end of October 2007. *Results:* At 24 months on treatment: Hemoglobin increased from 8.9 g/dL at baseline to 12.7 g/dL and platelets increased from 120x103/uL to 146x103/uL. Reduction of the splenic size was 74.5% and abdominal circumference was reduced from 89cm to 79cm. General clinical improvement with gain in body weight (from 33kg to 38.5 kg) was noted, as well as an improvement in both physical fitness and sense of wellbeing, as reported by the patient. No adverse effects or development of specific antibodies to velaglucerase alfa were observed up to 24 months on treatment. *Conclusion:* velaglucerase alfa, human cell line derived Gene-Activated" human glucocerebrosidase, was well-tolerated and demonstrated clinical activity in this patient with type 1 GD.

Postimmunization antibody responses to polysaccharide antigens in splenectomized and non-splenectomized patients with type 1Gaucher disease

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Host defense against encapsulated bacteria like Streptococcus pneumoniae largely depends on the production of anti-capsular polysaccharide antibodies and opsono-phagocytosis. Individuals with anatomical or functional hyposplenia or asplenia are at risk of acquiring severe infections by encapsulated microbes. We have immunized 15 type 1 Gaucher patients with the 23-valent pneumococcal polysaccharide vaccine Pneumovax 23. Six patients had been previously splenectomized. Enzyme-linked immunosorbent assay (ELISA) was used to measure serum antibodies to capsular polysaccharides (caps-PS) before and four weeks after immunization. Our studies showed that all the splenectomized and non-splenectomized Gaucher patients mounted an adequate antibody response with a titer increase of fourfold or more comparing to baseline titers. These data suggest normal antibody responses to polysaccharide antigens in splenectomized and non-splenectomized Gaucher patients.

Home therapy is safe for GA-GCB intravenous enzyme replacement therapy in patients with type 1 Gaucher disease

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Patients with Gaucher disease requiring continual intravenous therapy have, for more than 15 years benefited from the choice of venue for their health care, which has enabled patients or parents/carers to carry out treatments at their convenience. From the advent of enzyme replacement therapy (ERT) for Gaucher disease, because frequent infusions were burdensome for many patients and because it was appreciated that therapy would be chronic, home infusions were recommended (Zimran et al, 1993). As per the experience of hundreds of patients world-wide (Hughes et al, 2007), the safety and lack of adverse effects of the standard ERT further encouraged patients to elect home therapy. More recently, with the initiation of clinical trials with a new ERT for type 1 Gaucher disease, GA-GCB (Shire HGT, Cambridge MA), home

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therapy was made available within the context of an extension to the seminal trial and subsequently to patients on a switch-over trial from the standard ERT which hey had been receiving in the context of home therapy. Uninterrupted and safe home therapy for these patients facilitated participation in clinical trials that tend to continue into an extension phase prior to marketing approval. The current report includes the experience of three senior nurses who administer home therapy for a total, to date, of 17 patients for more than two years. The excellent safety profile and lack of adverse events related to infusions make the home therapy option ideal.

Persistent bone disease in adult type 1 Gaucher disease despite increasing doses of enzyme replacement therapy

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The most debilitating feature of Gaucher disease type I is skeletal disease, leading to chronic bone pain, pathologic fractures, avascular necrosis and bone crises. Although symptomatic bone disease is usually treated with high dose enzyme replacement therapy (ERT), >30% of patients do not show a clear improvement in bone marrow involvement. Whether it is useful to continue or increase high dose ERT in patients with ongoing bone disease is unknown. Therefore, we retrospectively compared adult GD patients with (group 2, N=12) and without (group 1, N=28) persistent symptomatic bone disease during ERT. At baseline, patients from group 2 had lower bodyweight and more severe disease. Dose was increased in 36% of patients from group 1 and 83% of patients from group 2. In the latter group, chitotriosidase and bone marrow fat fraction as assessed by QCSI showed a considerably better response after a dose increase in only two and none of the patients, respectively. The time to reach a QCSI of > 23%, and the time to reach a decrease in chitotriosidase levels of >80% were significantly slower in group 2 vs group 1 (median 132 vs 24 months, P=0.001, and median 172 vs 70 months, P=0.008). In conclusion, a subset of Gaucher type I patients experience ongoing bone disease despite increasing doses of ERT. Assessment of baseline characteristics and response parameters may provide a risk indication. Further dose increases are probably not effective, and dose decrease and alternative strategies should be considered. Sanctuary sites in the bone marrow, which were found in tissue from a surgically removed femur of a selected case, probably attribute to this phenomenon.

Post-marketing surveillance update for miglustat in type 1 Gaucher disease (GD1)

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Introduction: When the EMEA approved miglustat (Zavesca) for the treatment of patients with mild-to-moderate type 1 Gaucher disease (GD1) unsuitable for enzyme replacement therapy, they requested a post-marketing surveillance programme to monitor the tolerability and safety of Zavesca. The programme, called 'IS3', was initiated in March 2003. *Methods:* IS3 is a non-interventional, prospective, web-based programme conducted in accordance with standard of care practice and miglustat product characteristics. Data entry by participating physicians is voluntary. *Results:* As of June 1st 2007, 103 patients with GD1 prescribed miglustat in 48 centres across 11 European countries (mean [SD] age 45.1 [15.9] years 57.3% female) were included. The median (range) exposure to miglustat from the start of treatment in IS3 to discontinuation / data cut-off was 84.7 (3.7-211.6) weeks. At baseline, GD1 disease severity was classified mostly as mild or

moderate (76% and 22% of patients, respectively), though bone disease and neurological manifestations were present in 49/66 (74.2%) and 28/68 (41.2%) patients, respectively. Neurological safety signals were reported in 23 (23.3%) patients including two new cases of polyneuropathy with no clear relationship to miglustat treatment. 28/103 (27.2%) patients discontinued treatment. Adverse events most commonly associated with discontinuation were gastrointestinal disturbances (12/103 [11.6%] patients), two-thirds of which occurred during the first 6 months of therapy. *Conclusion:* These data represent 194 cumulative patient-years of post-marketing experience with miglustat in GD1, expanding previously existing data gained from clinical trials. No new safety findings were identified. IS3 is effective for monitoring miglustat safety in clinical practice.

Ambroxol is a Unique Pharmacological Chaperone for Human ²-Glucocerebrosidase and a Potential Treatment for Gaucher Disease

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Ambroxol, trans-4-(2-amino-3,5-dibromobenzyl)-amino-cyclo-hexanol, is an approved pharmaceutical with 30+ years of clinical experience. It has expectorant, anti-inflammatory, anti-oxidant, and anesthetic effects and is a Na+ and Ca++ channel and ionotropic glutamate receptor blocker. It increases surfactant secretion in the lungs and has been used to treat infant respiratory distress syndrome (IRDS) due to surfactant deficiency. Ambroxol, the active metabolite of bromexine, has superior pharmacokinetics, efficacy, and side effect profile than bromhexine, and had no major drug-related adverse events reported in long-term study (>2 years, 75 mg PO QD). In rats, Ambroxol is widely distributed in lung, liver, kidney and brain, and almost completely absorbed after oral or rectal administration. Oral availability = 70-80% after first-pass metabolism, renal excretion~80% and metabolites (mainly conjugates and biological inactive) are excreted via the biliary system, plasma protein binding ~90%, and the plasma t1/2 = 4-9 hrs with steady state achieved in ~2 days. Ambroxol attenuates acid ²-glucosidase (GCase) heat denaturation in vitro and promotes trafficking of GCase from the ER to the lysosome leading to increased lysosomal GCase activity. Ambroxol in contrast to isofagomine, possesses superior physicochemical and pharmacological properties. Physical studies demonstrate that Ambroxol stabilizes GCase, while and in contrast to isofagomine, allowing for the conformational flexibility of the enzyme. We hypothesize that stabilized, conformationally stable GCse may cleave glucosylceramide from lipid rafts in membranes more efficiently tha other pharmacological chaperones. We hypothesize that such conformational flexibility, will permit GCase to efficiently metabolize lipid raft membrane-associated glucosylceramide. Potentially, Ambroxol may become the therapy of choice for patients with all forms of Gaucher disease who have a "rescuable" and "enhanceable" mutation.

Low HDL cholesterol levels in type I Gaucher disease do not lead to an increased risk of cardiovascular disease

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HDL cholesterol (HDL-c) levels are abnormally low in type I Gaucher disease (GD) patients. A low plasma HDL-c concentration is an independent and important risk factor for the development of cardiovascular disease (CVD). The aim of this study was to determine whether GD is associated with premature

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atherosclerosis and whether this results in an increased incidence of CVD. Lipid profiles, apolipoproteins, and carotid artery intima-media thickness (cIMT) were analyzed in 40 type I GD patients, 34 carriers and 41 control subjects. cIMT is a non-invasive validated biomarker for the status of atherosclerosis and present and future cardiovascular disease risk. Data on cardiovascular events were obtained from patient files and indirect standardisation was used to compare the incidence in patients to that in the Dutch population. Compared to control subjects, patients showed decreased HDL (1.1 +/- 0.3 mmol/L) as well as mildly decreased LDL cholesterol levels (2.8 +/- 0.7 mmol/L), with an increased ApoB/ApoA1 ratio. In carriers, HDL-c levels were normal, but LDL-c levels were decreased (2.7 +/- 0.8 mmol/L). Mean cIMT measurements were not different in the three study groups. No increased incidence of myocardial infarction or cerebral stroke was found in GD patients. Our data show that the low HDL-c levels in type I GD patients do not result in an increased risk of CVD. This indicates that the relationship between low HDL-c levels and increased risk for CVD in the general population cannot be extrapolated to all conditions characterised by a low HDL-c level such as Gaucher disease.

Long term efficacy and safety of miglustat therapy in type 1 Gaucher disease. ZAGAL study

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Background and objective: Gaucher disease (GD) is the most common lysosomal storage disease. Miglustat ZAVESCA®, is a synthetic iminosugar which acts as an inhibitor of the enzyme glucosylceramide synthase (SRT). This therapy offers an alternative approach for GD based on the indirect effect reducing the burden of glycolipids delivered to the macrophage system after phagocytosis of formed blood cells. We present the results after more than 36 months on an everyday clinical use of oral therapy in type 1 GD patients. *Design and Methods:* The Spanish group on GD design a structured project named with the acronymus ZAGAL. It includes a set of recommendations in a structured protocol for collecting safety, efficacy and QoL data at 6, 12, 24, 36 months and beyond. The project's aim is to guarantee the safe and proper use of Zavesca in an everyday clinical use. *Baseline assessment:* complete clinical, analytical and imaging evaluation, detailed neurological exam and superficial electroneurogram in sural and peroneal nerve. Cognitive test and memory impairment screen for dementia assessment were used. A free lactose and low carbohydrate diet (FLLCD) were recommended and applied in first weeks on therapy. A SF-36 survey was evaluated after 24 months on therapy. *Results:* 47 GD patients (females 56.1%). mean age 44.4.y (range: 21-74), SSI 6.8(range: 2-9), spleen removal 9.5%, chitotriosidase activity 3,756 nM/mL.h (range 468-10,553), CCL18/PARC 533 (range 102-1,219). Ten patients were naïve to SRT, mean age: 46.7y. Thirty seven patients were included on SRT once stabilising their disease with Imiglucerase during a mean of 3.8 y(range:2-11), dose 30-60 U/kg, mean age 39.2 y mainly heterozygous for N370S. In February 2008 15 patients had completed 36 months on SRT, 26 patients 24 months and 10 naïve patients/37 switch 12 months. Response: all patients with anaemia improved haemoglobin concentration (mean 0.85 g/dL). Platelet count improved in patients with lowest values and it was maintained in patients with counts in normal limits. Chitotriosidase activity was maintained in switched patients and slightly decreased in naïve patients. The response was observed at 6 months on therapy and it is maintained after 24 months on therapy. In 7 naïve patients bone marrow MRI improvement was documented. No new symptoms were developed, three patient discontinued SRT due to poor compliance. Gastrointestinal disturbance appeared sporadically in five patients and became normal when they followed the FLLCD. The QOL analysis showed satisfactory results after two years on therapy *Conclusions:* In our experience type 1 GD patients with mild or moderate disease had a satisfactory clinical, analytical and bone-marrow response to SRT with scarcely adverse events. At six months in naïve patients the response is similar to that observed in clinical trials and in patients treated with Imiglucerase and remained stable at 24 and 36 months, with a satisfactory QOL. This study is partially supported by grants: FIS 06/1253, EC07_90737,FEETEG

Differential in vitro responses in inflammatory and immune cytokine production elicited by enzyme replacement therapies for Gaucher disease

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Gaucher disease is a lysosomal storage disorder resulting from a deficiency in the enzyme glucocerebrosidase within lysosomal compartments of macrophages in all tissues. The resultant abnormal function of these macrophages (Gaucher cells) leads to the disease manifestations and complications that include enlargement of the liver and spleen, secondary hematologic abnormalities and destructive bone disease. It has been reported that the pathogenesis of Gaucher disease is associated with the systemic production of pro-inflammatory cytokines and some clinical manifestations of Gaucher disease may be directly linked to the up-regulation of immune system mediators. In order to understand the potential impact of enzyme replacement therapy for Gaucher disease on pro-inflammatory cytokine production by human cells, normal human peripheral blood mononuclear cells (PBMCs) were treated with imiglucerase (Cerezyme®) or velaglucerase alfa (GA-GCB), at varying concentrations and for varying lengths of time and RNA and culture medium were collected. Transcriptional analysis of imiglucerase-treated cells revealed a dramatic up-regulation in the expression of genes involved in immune, inflammatory, apoptotic, and proliferation pathways. By comparison, velaglucerase alfa-treated cells only revealed an up-regulation in the expression of genes involved in proliferation. These results were confirmed by ELISA where imiglucerase treatment resulted in the increase of pro-inflammatory cytokines (IL-1b, IL-6, IL13, TNF). These cytokines were not elevated in response to velaglucerase alfa treatment. These findings suggest the need for additional studies to determine the nature of imiglucerase enhancement of pro-inflammatory cytokine production by human cells and if this elevation has clinical implications by impacting inflammatory cascades associated with the pathogenesis of Gaucher disease.

Progressive kyphoscoliosis in patients with chronic neuronopathic Gaucher disease

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Kyphoscoliosis is often found but rarely reported in patients with chronic neuronopathic Gaucher disease (CNGD). The aim of our cross-sectional study in CNGD patients is to determ age of onset, progression rate, risk factors, outcome and therapeutic aspects in the German patient cohort. Patients and results: In 16 patients CNGD was diagnosed in the age of 0 - 32 years. 14/16 suffered from kyphoscoliosis. Only in 2 patients with severe epileptic encephalopathy and early death kyphoscoliosis was not found. In homocygous L444P patients gibbus occurred in the 2nd year of life, others had later onset. Severity of kyphoscoliosis ranged from mild gibbus to severe fixed torsion of the thoracic scelet and restrictive lung function limitation in patients older than 20 years. Progression correlates with age, but not with detoriation of neurological signs or bone disease. Physiotherapy and corsets may slow down progression rate, but do not prevent severe kyphoscoliosis. Discussion: Progressive kyphoscoliosis in CNGD patients is leading to severe assymetric posture disability and limitation of lung function. The severity of kyphoscoliosis contributes to the overall disease burden and should be part of a disease-score like the Davies-Score. The pathogenesis is so far unknown. Therapeutic strategies have to be investigated.

Time interval between diagnosis of type 1 gaucher disease and initiation of enzyme therapy and splenectomy are determinants of avascular necrosis

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Objective: Treatment of type 1 GD (GD1) is initiated at varying intervals following diagnosis. This study assessed the effect of elapsed time from diagnosis to initiation of alglucerase/imiglucerase treatment on the subsequent risk of developing avascular necrosis (AVN), the principal bone manifestation of GD1. The secondary aim identified other determinants of AVN. Methods: All alglucerase/imiglucerase-treated patients with GD1 enrolled in the ICGG Gaucher Registry without documented AVN prior to initiation of therapy were included. Incidence rate for the first occurrence of AVN after starting therapy was calculated according to time intervals from diagnosis to initiation of alglucerase/imiglucerase. Other risk factors investigated included spleen status, GBA genotype, age at therapy initiation and enzyme dose. *Results:* 2,700 patients met the inclusion criteria. Among GD1 patients who began alglucerase/imiglucerase 2 or more years after diagnosis, the incidence rate of AVN was 16.6 per 1,000 person-years. Patients with an interval to start of treatment less than 2 years had a lower rate of AVN (incidence rate 8.1 per 1,000 person-years incidence rate ratio <2 years vs. >2 years 0.49, 95% confidence interval 0.35 - 0.68). Patients with antecedent splenectomy (total or partial) had a higher incidence rate of AVN, regardless of the timing of treatment initiation: e2 years between diagnosis and initiation of therapy, 26.6 per 1,000 person-years <2 years between diagnosis and start of therapy, 29.7 per 1,000 person-years. *Conclusion:* With an interval of more than 2 years between GD1 diagnosis and initiation of alglucerase/imiglucerase treatment, patients have increased risk of post-treatment, osteonecrosis.

Enhanced Abundance and Processing of Cathepsin S: a potential Biomarker of Gaucher disease

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Macrophages express cathepsin S, a cysteine proteinase. Lysosomal processing of 39kD pro-cathepsin S yields the mature active 28kD isoform - which is abundant in Gaucher spleen. Since pro-cathepsin S is autoactivated at neutral pH, extra-lysosomal cathepsin S may contribute to the disease process. Time-resolved fluorescence immunoassays were used to determine human total cathepsin S (pro, mature, and cystatin-complexed forms) and pro-cathepsin S in 25 Gaucher sera from patients aged 17-62 years (mean 40) before enzyme treatment and in age-matched healthy control sera. Cathepsin S isoforms were also determined in sera from 11 patients receiving enzyme therapy for 1-10 years. Total cathepsin S in Gaucher sera was elevated 10-fold without overlap (median 116 ng/mL, range 28-301 ng/mL), compared with control sera (median 10.4 ng/mL, range 5-17 ng/mL $p<0.001$). The mean proportion of pro-cathepsin to the mature isoform in the serum of untreated Gaucher patients (7.7%) was lower than that in healthy subjects (34%, $p<0.05$). Pro- and mature cathepsin S isoforms were reduced by therapy in all 11 patients - and pro-cathepsin processing was diminished. After 5 years treatment in 6 patients, total cathepsin S (median 199 ng/mL, pro-cathepsin S proportion - 5.4%) was significantly decreased with increased proportion of pro-cathepsin S (median 31.5ng/mL, 17% respectively, $p<0.05$). Conclusion: Increased total cathepsin S concentrations in Gaucher serum are associated with enhanced cleavage to the mature isoform, probably due to pathological activation of the Gaucher cell, enzyme therapy decreases cathepsin S release and corrects the processing abnormality.

Epileptic encephalopathy in patients with chronic neuronopathic Gaucher disease

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Myoclonic epilepsy leading to progressive encephalopathy is known as severe manifestation of chronic neuronopathic Gaucher disease (CNGD). The aim of our cross-sectional study is to analyze clinical features, age of onset, risk factors, outcome as well as anticonvulsant medication in the German patient cohort. Patients and results: In 16 patients CNGD was diagnosed in the age of 0 - 32 years. 6/16 suffered from myoclonic epilepsy. Myoclonus proceeded grand mal seizures 1-2 years. Risk factors for epileptic encephalopathy are splenectomy, primary neuronopathic Gaucher disease manifestation and genotype not homocygous L444P. In CNGD patients with epileptic encephalopathy progressive neurodegeneration and early death was seen, whereas the other patients with CNGD are stable during childhood and adolescens. Grand mal seizures can be controlled with valproat alone or in combination. However, myoclonus and progressive neurodegeneration could not prevented. ERT is ineffective regarding epileptic encephalopathy. High cortical waves in SSEP or VEP and increasing latency of wave I-V in BAER are electrophysiological hints for epileptic encephalopathy. *Discussion:* Early anticonvulsant therapy of myoclonus and seizures is necessary to preserve fine motor function and autonomy of life, but the effect is particulary with regard to the course of progressive encephalopathy very limited. At present the prognosis of epileptic encephalopathy in CNGD patients is due to insufficient therapeutic options poor. New therapeutic strategies are needed.

Bone densitometry usefully in the evaluation of bone disease in type 1 Gaucher patients. A preliminary comparative study

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Background: Skeletal manifestations in Type 1 Gaucher disease (GD1) patients are characterized by a high incidence of osteopenia that progresses with age and could be related with the severity of the disease. Dual-Energy X-ray Absorptiometry (DEXA is the standard technique to evaluate bone mineral density (BMD), that procedure measures bone density in the axial skeleton and femoral neck. Nevertheless other procedures to study the bone characteristics based in physical principles as Dual Fotonic absortiometry (DFA) can measure the amount of mechanical energy transmitted by the bone, as well as their transmission speed (BUA). Calcaneus has been the bone most routinely used for this exam because is a flat, symmetrical bone, that allows for precise and repeated measurements. its composition is 95% trabecular although trabecular bone only constitutes 20% of the bone mass it represents 80% of its metabolism due to a more important vascularization. Both procedures are useful to obtain information about the risk of developed bone complications. Aims: In order to evaluate bone disease indicators, we have planned a comparative analysis in GD1 by both techniques to exam individual variability in bone mineral density and to study the response to therapy. *Patients and Methods:* We have design a comparative blind study to determine Z-score by conventional DEXA versus calcaneus DFA. The advantages/disadvantages of each procedure and the correlation of results with the clinical characteristics, follow-up, response to therapy, S-MRI, BMB score and subrogate biomarkers has been evaluated. The study has been performed in 25GD1 adults in different time of follow-up: at diagnosis, before start therapy and five years after therapy. The results have been integrated with genotype, clinical characteristics and differences in bone disease related to therapy. *Results:* A significant correlation has been observed in bone density parameters obtained in the BMD study in GD1 patients. Low bone density parameters are correlated with high S-MRI scores. The final results will be presented in the meeting *Comments:* More experience is needed in order to precisely define the role of DFA in the management of GD patients, but there is no doubt that DFA provides additional bone

information to BMD obtained with DEXA. On the other hand, the lack of radiation hazard, its portability and lower cost favors its inclusion into the diagnostic armamentarium of GD.

Hematological malignancies in type 1 gaucher disease: Diagnosis and treatment challenge

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Objective: Coexistence of immunoproliferative disorders and Type I Gaucher disease (GD) had been reported in small groups of patients. To evaluate the frequency, diagnostic measures and treatment approach of concomitant hematological malignancies and Type I GD. *Methods:* The clinical and laboratory data of cohort of 124 Type I GD patients followed at the Hematology department in the Rambam medical center in Haifa, Israel were evaluated for hematological malignancies. *Resutlts:* Among 124 GD patients 14 (11.2%) presented with hematological malignancies: 8/14 were diagnosed with malignant lymphoma, 4/14 with multiple myeloma and 2/14 presented myelodyplastic syndrome. In four patients monoclonal gammopathy of unknown significance was detected. In 4/14 (28%) the Type I GD was diagnosed during evaluation and staging of the hematological malignancy. Two of the GD patients presented diagnostic difficulties due to the massive infiltration of the bone marrow by Gaucher cells, requiring immunohistochemical staining, immunophenotyping and PCR studies. The concurrence of GD and hematological malignancies raised the question of concomitant chemotherapy and enzyme replacement therapy (ERT) in these patients. *Conclusions:* A high frequency of hematological malignancies was found among GD patients. The association of GD and hematological malignancies required careful diagnostic measures and was challenging regarding treatment by standard chemotherapy regimens and ERT.

Management of patients with Gaucher's disease (GD) in a French centre

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Background: In the French Gaucher Registry 470 patients are actually registered: 270 are treated by Enzyme Replacement Therapy (ERT) and 20 by Substrate Reduction Therapy (SRT). *Objectives:* We report here a French experience of a single centre following patients with Gaucher disease. Methods Patients were included in this cohort since 1991. Clinical, biological and radiological data has been reported restrospectively until 1 may 2007. Evolution of biomarkers on ERT was schematised with and without treatment. *Results:* Seventy-three patients were included. One patient died of pulmonary hypertension during follow-up. Forty-two patients (58%) were women and 31 patients (42%) were men. Median age of diagnosis was 16 years. First symptoms started at the mean age of 10 years. The median time of medical follow-up from diagnosis was 21 years [range: 0 - 67 years]. Bone marrow aspiration brought the diagnosis of GD for 59% patients. Splenectomy was a diagnosis method for 8 cases (14%). The diagnosis was confirmed in all patients by assaying glucocerebrosidase activity. All the patients had a phenotype 1, except 4 patients with a phenotype 3. Genotype was known for 57 patients: 13 were N370S/N370S and two were L444P/L444P. Overall 16 patients (28%) had splenectomy and 42 patients (58%) had bone disorder (bone crisis, infarcts, osteonecrosis and pathological fracture) during evolution. Sixty-three patients were treated: 62 with ERT and one with SRT. Evolution of biomarkers was helpful for the management of treatment. *Conclusion:* Our data suggest management of GD in a reference centre is improving.

Long-term bone effect of enzyme replacement therapy (ERT) in two splenectomized children with type 1 Gaucher disease (GD)

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Childhood manifestations of type 1 GD are usually predictive of more severe phenotype, and bone diseases are more severe in splenectomized patients. We have monitored skeletal and visceral pathology between 1988 and 2008 in two boys with type 1 GD, who were splenectomized at ages of 20 (Patient 1) and 22 month (Patient 2). Mutation analysis revealed G377S/L444P in Patient 1 and homozygosity for the L444P mutation in Patient 2. Homozygosity for L444P is usually associated with type 3 GD, but this patient exhibited no signs of central nervous system disease. Erlenmeyer flask deformity was the only bone abnormality in each patient at 3 years of age. Fractures, deformities, and destruction of one or more long tubular bones, and compression fractures of 3 and 4 vertebral bodies in Pt1 and Pt2, respectively, developed between ages of 5 and 6. ERT became available when the boys were 9 and 8 years old and was started with a dose of 15 IU/kg biweekly. At ages 12 and 11, bone crises (one episode in Pt 1 and three in Pt2) were diagnosed and the enzyme dose was increased to 30 and 60 U/kg/2 weeks. Bone marrow infiltration started to improve two years after ERT was started, and almost normal bone marrow content could be seen in femora after 8 and 11 years ERT. Bone crises did not recur. Measurable bone remodeling developed in the humerus and the proximal part of the femur 5-6 years after initiation of ERT. However, two thoracic vertebrae in each patient remained extremely flat and sclerotic. This study suggests that compression fractures of vertebral bodies may not be restored even by long-term ERT in patients with GD. We also found that the Erlenmeyer flask deformity was not influenced by ERT in these patients.

Modelling the evolution of biomarkers under treatment in Gaucher Disease

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Background: Gaucher Disease (GD) involves an increase in biomarkers such as ferritin, chitotriosidase, tartrate-resistant acid phosphatase (TRAP) and angiotensin-converting enzyme (ACE), and a decrease in platelets. Generally, under treatment inverse evolution of these markers is observed. *Objectives:* To quantify the evolution of these 5 biomarkers when patients receive treatment by Enzyme Replacement Therapy with Imiglucerase or Alglucerase and to analyse the influence of several covariates on the evolutions. *Methods:* Analysis is based on 62 patients whose data had been collected retrospectively in Beaujon Hospital, Clichy, France. Median follow up is 21 years. For platelets there are 897 observations (median of 10 visits by patients). We use linear mixed models to analyse evolution of biomarkers taking into account repeated measurements within patients. Five covariates are tested in the models: splenectomy, diagnosis date (before/after 1991), genotype (N370S-N370S/others), age at diagnosis (before/after 15 years old) and sex. *Results:* Evolution of platelets under treatment is a linear increase whereas evolution of ferritin, chitotriosidase, TRAP and ACE are exponential decreases, so logarithmic transformations are used in the model. For platelets we found a significant effect of splenectomy on baseline level ($p<0.0001$) and growth ($p=0.0007$). Splenectomy and age at diagnosis have influence in TRAP decrease and baseline diagnosis date has an influence in ACE baseline splenectomy and genotype have influence in chitotriosidase decrease and baseline. *Conclusion:* Modelling is a powerful tool to quantify the evolution of biomarkers under treatment and to test for the influence of covariables. Relationships with occurrence of clinical event should be studied.

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A report from the international collaborative gaucher group (ICGG) gaucher registry

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Objective: To report the latest data on patients with Gaucher disease (GD) enrolled in the ICGG Gaucher Registry. *Methods:* Data from all patients enrolled in the ICGG Gaucher Registry from 1991 through 2007 were analyzed. *Results:* As of December 31, 2007, 4,936 GD patients were enrolled in the ICGG Gaucher Registry by 772 physicians in 60 countries. The countries with the largest numbers of patients were the United States (36%), Israel (14%) and Brazil (10%). The majority of patients were diagnosed with GD between 4 and 30years (mean age, 19y). The most common genotypes were N370S/N370S (31%), N370S/L444P (16%), N370S/Rare Allele (13%), and N370S/? (11%). The most frequent genotype for patients with neuronopathic GD was L444P/L444P (68%). At diagnosis, anemia was reported in 37% of patients and moderate to severe thrombocytopenia in 60%. Splenomegaly was reported in 86% of patients (>5 multiples of normal, MN) and hepatomegaly in 65% (liver volume >1.25 MN). Bone pain was present in 34% of patients and radiologic bone disease was reported in 83%. Long-term treatment with imiglucerase resulted in improved haematological parameters, decreased visceral involvement, decreased bone pain and abolition of bone crises. *Conclusions:* For GD and other rare "ultra-orphan" diseases, a large longitudinal international disease registry provides the best means to investigate the natural history of the disease and the long-term effects of therapy. The strength of the ICGG Gaucher Registry data is the inclusion of a large, worldwide patient population with long periods of follow-up data, allowing for studies not otherwise possible.

An improved high-throughput multiplex enzyme assay to screen for lysosomal storage disorders in dried blood spots

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Background: Publication of the multiplex enzyme assay for Pompe disease, Fabry disease, Gaucher disease, Niemann-pick disease types A and B, and Krabbe disease engendered interest in its application in newborn screening (NBS). We have modified the assay into a robust, high-throughput assay for use in screening laboratories. *Methods:* The enzyme reaction conditions and procedures for the assay were optimized, including the concentrations of substrate (S) and internal standard (IS), assay cocktail composition, sample clean-up procedure and mass spectrometer operation. The S and IS for each enzyme were premixed and bottled at the optimized molar ratio to simplify assay cocktail preparation. Using the new S/IS ratio, the modified assay was validated following CLSI guidelines. Stability of the S, IS, and the assay cocktails were investigated. Dried blood spots from healthy adults (149), newborns (100) and LSD patients (60) were tested using the modified assay. Results In our study, the median activity of adults was generally increased 2-3 fold relative to the original method and consequently, the assay achieved high precision and robustness. In the multiplex format, each of the five modified enzyme assays showed an unambiguous differentiation between normal (adults and newborns) and the corresponding disease-specific samples. *Conclusion:* The modified multiplex enzyme assay, using premixed S and IS, is ready to be transferred to other laboratories.

High-throughput Screening Assay of Gaucher Disorder by Dried Blood Spots

Olivova P., Cullen E., Titlow M., Kallwass H., Barranger J., Keutzer J., Zhang K.
Genzyme Corporation

Introduction: Gaucher disease is an inherited recessive autosomal metabolic defect due to a deficiency of the lysosomal enzyme b-glucocerebrosidase(GBA, EC 3.2.1.45). The enzyme substrate, glucocerebroside, accumulates in the lysosomes of cells in the monocyte/macrophage system, predominantly in the liver, spleen, and bone marrow. Osteoarticular manifestations are often inaugural and contribute much of the morbidity and disability associated with Gaucher disease1. The diagnosis of Gaucher disease is confirmed by measuring GBA activity in peripheral blood leukocytes however, many patients are misdiagnosed or remain undiagnosed because Gaucher disease is rare and patients can have heterogeneous symptoms. A simple screening method would increase detection rate and allow for early implementation of therapy when needed to prevent the serious complications of Gaucher disease. *Methods:* We have developed a high-throughput and reliable screening assay for measuring GBA activity in dried blood spots (DBS) based on the method of Chamoles et al.2. The principle of the assay is detecting fluorescence of 4 Methylumbelliferone (4-MU) generated in an acidic pH within 20 hours as a product of the fluorogenic substrate 4 Methylumbelliferyl-² D glucopyranoside (4-MU-²-Glu) cleavage. Enzyme from DBS samples is extracted in a 96-well plate. Conduritol B Epoxide (CBE), a specific and irreversible inhibitor, is used to evaluate the contribution of other ²-glucosidase isoenzymes to the measured activity. The difference between activities in the absence and presence of CBE is used to quantitate GBA activity. *Results:* Following the procedure outlines in CLSI (Clinical and Laboratory Standards Institute) guideline found in EP05, the estimate of assay precision was determined by testing six normal samples (high, middle, and low GBA activity) in quadruplicates on two plates per day for 5 days by 2 operators. The overall assay variation is less than 15%. The limit of detection was determined to be 1.3 pmol/punch/h. We measured GBA activity in DBS samples from 15 previously diagnosed Gaucher disease patients and 185 normal control samples. The mean GBA activity for normal control samples was 10.8 pmol/punch/h (minimum 5.6 maximum 23.8 pmol/punch/h mean activity 10.3 - 11.2 pmol/punch/h). Two Gaucher disease samples were below the LOD the maximum was 4.0 pmol/punch/h). *Conclusions:* The results demonstrate that the assay is sensitive enough to differentiate DBS from patients with Gaucher disease from normal controls. The DBS assay's speed, high-throughput, and low cost make it an ideal method to screen for Gaucher disease. The applicability of this method for diagnosing Gaucher disease remains to be determined.

Up to 42-Months on Treatment: Open-Label Phase I/II Long-Term Study of Enzyme Replacement Therapy (ERT) with velaglucerase alfa in Patients with Type 1 Gaucher Disease

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Aim: To evaluate the long-term safety and clinical activity of velaglucerase alfa (Gene-Activated" human glucocerebrosidase), a novel ERT for patients with type 1 Gaucher disease. *Background:* velaglucerase alfa is produced in a well-characterized human cell line using proprietary gene-activation technology and has an identical amino acid sequence to the naturally occurring human enzyme. *Methods:* Ten of 11 patients who completed the Phase I/II study enrolled in the long-term extension study. One patient since discontinued treatment for reasons unrelated to velaglucerase alfa. During the first 12 months of treatment with velaglucerase alfa patients received 60U/kg velaglucerase alfa every other week. At or after Month 12, all patients qualified (based on the Therapeutic Goals for ERT in Type 1 Gaucher disease published in Seminars in Hematology, 2004) to begin a step-wise dose reduction from 60U/kg to 45U/kg (13 weeks) and then to 30U/kg. *Results:* The preliminary data up to Month 42 on treatment with velaglucerase alfa are included in this abstract. Patients were dose reduced from the original dose of 60U/kg to 30 U/kg without statistically significant changes in clinical parameters. Safety results up to Month 42: velaglucerase alfa has continued to be generally well tolerated. To date, no drug-related serious adverse events have been reported. Most AEs were mild to moderate and include arthralgia, back pain, headache, abdominal pain, and pharyngolaynal pain. Notably, no patient has developed antibodies to velaglucerase alfa up to Month 42. Efficacy results at the last time point for which data are available: At Month 42, there were statistically significant increases in hemoglobin from baseline (mean increase = 2.18g/dL mean percent change of 19.0% p=0.004) and in platelet counts from baseline (mean increase = 82.1 x 103/mm3 mean percent change of 149.8% p=0.004). At Month 33, MRIs showed statistically significant decreases in the mean percent change of spleen and liver volume from baseline (by 72.3% and 29.6%, p=0.008 and p=0.004 respectively). Similarly, at Month 30, there were decreases in the mean percent change from baseline in the biomarkers chitotriosidase (by 83.5% from baseline) and CCL18 (by 57.2% from baseline). Statistically significant mean increases from baseline in the main study were observed in both hemoglobin and platelet counts by Month 3 and were maintained through Month 42. *Conclusion:* velaglucerase alfa was generally well tolerated and demonstrated clinical activity in disease parameters in these adult patients with type 1 Gaucher disease. These results have led to the development of Phase III clinical trials that are currently enrolling adults and children. The Phase III clinical trials will further evaluate velaglucerase alfa in both treatment-naïve patients as well as patients currently receiving imiglucerase. www.clinicaltrials.gov, keyword: velaglucerase alfa or GA-GCB

8TH EUROPEAN WORKING GROUP ON
GAUCHER DISEASE (EWGGD) MEETING
JUNE 4–7, 2008

Company papers in 'Science and industry' session

From a small miracle to a small molecule and beyond: Genzyme's commitment to the Gaucher community

Moscicki Richard
Chief medical officer, Genzyme Corporation

When enzyme replacement was first being considered as a treatment for glucocerebrosidase deficiency, few realized the degree of the benefits that this revolutionary treatment would offer patients with Gaucher disease. Initially produced from placental material as Ceredase®, the recombinant enzyme commenced production in 1989. In 1994 and 1997, Cerezyme® was approved by the FDA and the EMEA respectively. It is now available in more than 80 countries worldwide and over 5,000 patients receive therapy with Cerezyme. This represents over 40,000 patient years of follow up.

The extensive experience from the ICGG Registry has provided valuable information concerning the natural history of untreated Gaucher disease and continued information on treated Gaucher disease. Thus, valuable answers to many questions regarding assessment of disease state and expected response at varied doses were provided.

Life expectancy and quality of life have been shown to be reduced in Gaucher disease. Quality of life is severely affected by bone disease, and at diagnosis, over 80% of patients have radiological evidence of skeletal manifestation of the disease. Assessment and monitoring guidelines and therapeutic goals, establishing the expected timeframe and level of response to Cerezyme treatment for each affected organ system, have been developed. Bone response to Cerezyme, the impact of dose, and of alternative administration schedules have recently been further elucidated.

The ICGG Gaucher Registry and the International and European Cerezyme access programs, which provide Cerezyme to patients in countries where it is not commercially available, represent Genzyme's corporate commitment both to the Gaucher community as a whole and to the individual with Gaucher disease.

Cerezyme has fulfilled most needs in the Gaucher community. However, some issues remain, most importantly CNS disease. Thus, despite having the gold standard treatment in Gaucher disease with Cerezyme, Genzyme is continuing to pursue new and innovative therapies to address these issues. Research into new therapies is ongoing, such as Genzyme's development of a small molecule which is highly selective and proving to be efficacious in inhibiting glucocerebroside substrate production. CNS administration of enzyme is actively being explored. Genzyme is also pursuing gene therapy as a final solution for lysosomal storage diseases.

In summary, Cerezyme has been proven to be very efficacious with a well documented safety profile and over the last 15 years has radically transformed the lives of patients with Gaucher disease, making it possible for them to live the lives they have chosen for themselves. Genzyme is committed to further improving the knowledge about, and treatment for, patients with Gaucher disease.

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Novel Enzyme Replacement Therapy for Gaucher Disease: On Going Phase III Clinical Trial with Recombinant Human Glucocerebrosidase Expressed in Plant Cells

Almon-Brill Einat[1], Shaaltiel Yossef[1], Galili Gad[4], Chertkoff Raul[1], Hashmueli Sharon[1], Galun Eithan[3], Aviezer David[1], Zimran Ari[2]
[1]Protalix Biotherapeutics, Carmiel, Israel; [2]Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel; [3]Goldyne Savad Institute of Gene Therapy, Hadassah Hebrew University Hospital, Jerusalem,Israel; [4]Plant Sciences, Weizmann Institute of Science, Rehovot, Israel

Gaucher Disease, characterized by glucocerebrosidase (hGCD) deficiency, provokes glucosylceramide accumulation in cellular lysosomes. Disease clinical pathology includes anemia, thrombocytopenia splenomegaly, skeletal pathology and pulmonary hypertension/infiltration. Current therapy uses mammalian based production of recombinant glucocerebrosidase for enzyme replacement therapy (ERT) that requires post-expression glycan remodeling for exposing mannose structures, required for intake by Macrophages Protalix has developed a propriety plant cell expressed active form of recombinant human glucocerebro-sidase (prGCD). The unique protalix technology enables control of glycosylation pattern and consistency through targeting to specific plant cell organelles. Hence, prGCD has intrinsic exposed mannose residues and demonstrates batch to batch consistency. prGCD exhibits similar crystal structure and biological activity to that of the currently used CHO expressed Cerezyme. Preclinical toxicology studies showed no treatment-related adverse events, no neutralizing antibodies and no clinical findings. Phase I safety clinical trial showed that prGCD administered intravenously in sequential doses (15, 30 and 60 units/kg) was well tolerated, all tests being within normal ranges, with no treatment related adverse events. Pharmacokinetic analysis demonstrated a prolonged half life. All immunological specific tests were within normal ranges with no significant immune reaction or production of anti-prGCD antibodies. An international multi-center Phase III Pivotal trial is currently ongoing under FDA Special Protocol Assessment approval where 30 untreated patients will be administered with 30U/kg or 60U/kg per infusion over 9 months. Following a completion of the protocol, patient are offered to enter an Extension study.

Differential in vitro responses in inflammatory and immune cytokine production elicited by enzyme replacement therapies for Gaucher disease

Martini P.[1], Onderdonk A.[2], Concino M.[1], Tzianabos A.[1], Robinson G.[1]
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Gaucher disease is a lysosomal storage disorder resulting from a deficiency in the enzyme glucocerebrosidase within lysosomal compartments of macrophages in all tissues. The resultant abnormal function of these macrophages (Gaucher cells) leads to the disease manifestations and complications that include enlargement of the liver and spleen, secondary hematologic abnormalities and destructive bone disease. It has been reported that the pathogenesis of Gaucher disease is associated with the systemic production of pro-inflammatory cytokines and some clinical manifestations of Gaucher disease may be directly linked to the up-regulation of immune system mediators. In order to understand the potential impact of enzyme replacement therapy for Gaucher disease on pro-inflammatory cytokine production by human cells, normal human peripheral blood mononuclear cells (PBMCs) were treated with imiglucerase (Cerezyme®) or velaglucerase alfa (GA-GCB), at varying concentrations and for varying lengths of time and RNA and culture medium were collected. Transcriptional analysis of imiglucerase-treated cells revealed a dramatic up-regulation in the expression of genes involved in immune, inflammatory, apoptotic, and proliferation pathways. By comparison, velaglucerase alfa-treated cells only revealed an up-regulation in the expression of genes involved in proliferation. These results were confirmed by ELISA where imiglucerase treatment resulted in the increase of pro-inflammatory cytokines (IL-1b, IL-6, IL13, TNF). These cytokines were not elevated in response to velaglucerase alfa treatment. These findings suggest the need for additional studies to determine the nature of imiglucerase enhancement of pro-inflammatory cytokine production by human cells and if this elevation has clinical implications by impacting inflammatory cascades associated with the pathogenesis of Gaucher disease.

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NOTES

Abbreviated Summary of Product Characteristics

Cerezyme® 200 U / Cerezyme® 400 U: powder for concentrate for solution for infusion (imiglucerase).
Prescription only medicine.

Product composition: Each vial of Cerezyme contains 200 U or 400 U of imiglucerase, the recombinant form of the natural form of human ß-glucocerebrosidase, and the following excipients: mannitol, sodium citrate, citric acid monohydrate and polysorbate 80.
Cerezyme should be reconstituted with water for Injections and further diluted in 0.9% sodium chloride intravenous solution.

Indication: Cerezyme is indicated for use as long-term enzyme replacement therapy in patients with a confirmed diagnosis of non-neuronopathic (Type 1) or chronic neuronopathic (Type 3) Gaucher disease and who exhibit clinically significant non-neuronological manifestations of the disease.
Contraindications: hypersensitivity to the active substance or to any of the excipients.

Dosage and administration: Therapy should be directed by physicians knowledgeable in the management of Gaucher disease. Initial doses of 60 U/kg of body weight once every 2 weeks have shown improvement in haematological and visceral parameters within 6 months of therapy and continued use has either stopped progression of or improved bone disease. Administration of doses as low as 2.5 U/kg of body weight three times a week or 15 U/kg of body weight once every 2 weeks has been shown to improve haematological parameters and organomegaly, but not bone parameters. The reconstituted and diluted preparation is administered by intravenous infusion over 1 to 2 hours.

Precautions:
Hypersensitivity: IgG antibodies to imiglucerase are formed in approximately 15% of the treated patients. It appears that patients will rarely develop antibodies to Cerezyme after 12 months of therapy. Patients with antibody to Cerezyme have a higher risk of hypersensitivity reactions.

Pulmonary hypertension: Pulmonary hypertension is a known complication of Gaucher disease. It has been observed both in patients receiving and not receiving enzyme replacement therapy. No causal relationship with enzyme replacement therapy has been established. Patients with respiratory symptoms should be evaluated for the presence of pulmonary hypertension.

Interaction: Interactions between Cerezyme and other medicinal products have not been studied. Other forms of interactions such as with food are unlikely.

Pregnancy and lactation: It is not known whether Cerezyme can affect reproductive capacity, or cause foetal harm when administered to a pregnant woman, or is excreted in human milk. Therefore, caution should be exercised when Cerezyme is administered to pregnant or nursing women.

Undesirable effects: In a small number of patients undesirable effects have been reported which are related to the route of administration: discomfort, pruritus, burning, swelling or sterile abscess at the site of venipuncture. In approximately 3% of the patients symptoms suggestive of hypersensitivity have been noted, like pruritus, flushing, urticaria/angioedema, chest discomfort, tachycardia, cyanosis, respiratory symptoms and paraesthesia. Hypotension associated with hypersensitivity has also been reported rarely. Additional undesirable adverse effects have been reported in a limited number of patients: headache, dizziness, nausea, vomiting, abdominal cramping, diarrhoea, rash, arthralgia, fever, rigors and fatigue.

Overdose: No case of overdose has been reported

Medical or healthcare professionals are encouraged to register non-neuronopathic (Type 1) and chronic neuronopathic (Type 3) Gaucher patients in the 'ICGG' Gaucher Registry to enhance the understanding of the disease and evaluate the effectiveness of the treatment.

Read the full prescribing information before using the product.
Status SmPC 09/2007.

Marketing authorisation holder: Genzyme Europe B.V., Gooimeer 10, 1411 DD Naarden, The Netherlands.
EU/1/97/053/001-005

genzyme

Cerezyme.
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Innovation – Dedicated to Patients

Research
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Actelion Ltd is a biopharmaceutical company with its corporate headquarters in Allschwil/Basel, Switzerland. As a leading player in innovative science related to the endothelium – the innermost cell layer of blood vessels separating tissue from the blood stream – Actelion focuses on the discovery, development and marketing of innovative drugs for significant unmet medical needs. Actelion shares are traded on the SWX Swiss Exchange (ticker symbol: ATLN).

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ACTELION

Amicus Therapeutics

Amicus is a clinical-stage biopharmaceutical company developing a new class of drugs called pharmacological chaperones. This novel approach has the potential to improve treatment options for individuals and families with a range of human genetic diseases.

The company's three most advanced product candidates are experimental treatments for Fabry disease, Gaucher disease, and Pompe disease.

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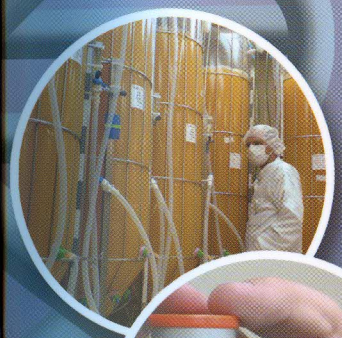
Protalix Biotherapeutics

Changing the way therapeutic proteins are made

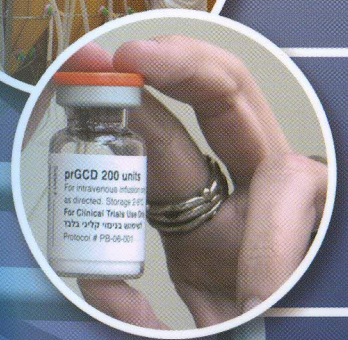
Protalix, an advanced clinical stage biopharmaceutical company, focused on the development and commercialization of recombinant therapeutic proteins based on its unique proprietary plant cell culture system – ***ProCellEx***™.

Demonstrated feasibility through expression of enzymes, cytokines, monoclonal antibodies, hormones and vaccines.



Advantages of the ***ProCellEx***™ system:

- Cost effectiveness and rapid scalability
- Free of any mammalian derived components
- Uniform Glycosilation patterns

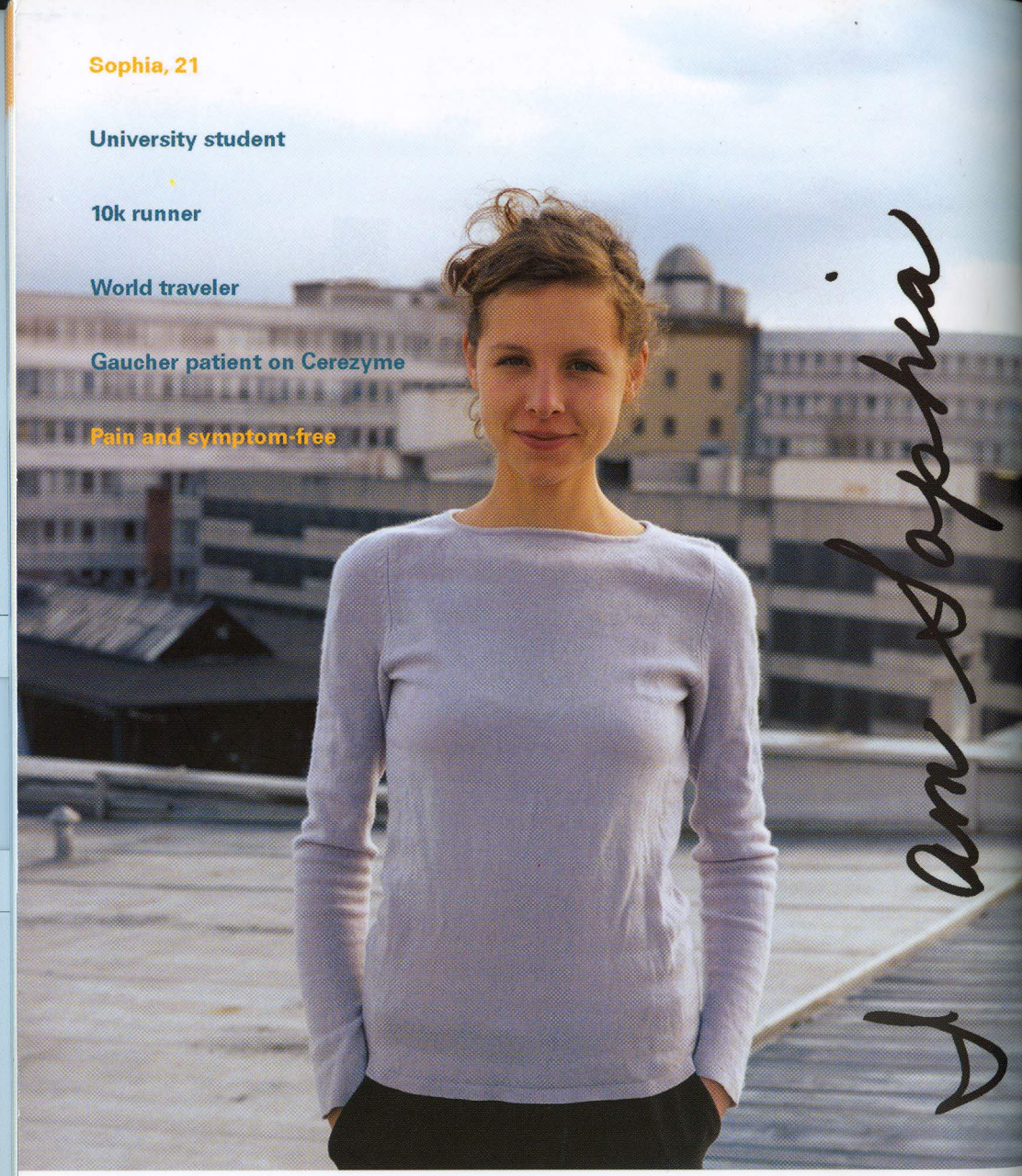


Lead product - ***prGCD*** - Glucocerebrosidase for Gaucher Disease is currently in Phase III clinical trial.

Publicly traded on the American Stock Exchange : PLX

Design by Tamarindi

www.protalix.com



Cerezyme – the gold standard of care – can reverse the diverse symptoms of Gaucher disease and can prevent long term complications such as bone disease when dosed appropriately.[1]

Cerezyme significantly improves overall quality of life and lets patients and their families live the lives they've chosen...for themselves.[2]

Cerezyme.
imiglucerase
Be yourself

Please read detailed Cerezyme Summary of Product Characteristics by visiting http://www.emea.europa.eu/humandocs/Humans/EPAR/cerezyme/cerezyme.htm

[1] Mistry P. et al. Am J Hematol 2007 Aug;82(8):697-701 [2] Weinreb N. et al. Clin Genet 2007 Jun;71(6):576-88

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