11TH EWGGD HAIFA

The European Working Group on Gaucher Disease

Proceedings of the 11th European Working Group on Gaucher Disease (EWGGD)

June 26th-28th 2014

Haifa | Israel

Published by the
European Gaucher Alliance
on behalf of the EWGGD

www.ewggd2014-israel.com

EWGGD
European Working Group on Gaucher Disease

Dear friends

As chairpersons of the association, Dr. Helen Michelakakis and myself would like to thank the EGA for collecting the essential information on the Haifa 2014 EWGGD workshop in this comprehensive brochure. It was meticulously arranged by the great team around the EGA and it is worth being read in total.

By the end of June 2014, the 11th Workshop of the EWGGD (European Working Group on Gaucher Disease) has taken place at the great Carmel site in Haifa, Israel´s most northern harbour and the city with the famous Bahá'í Gardens. You may believe it or not, but I visited with this site by chance with a good friend of mine Ari Zimran in 2009 and I thought it would be most desirable to reconvene here for the purpose of a scientific meeting. So, we were very happy it worked out.

This time, the meeting was hosted by the Department of Hematology at Rambam University and intellectual coverage was provided by the Technion - Israel Institute of Technology's Ruth and Bruce Rappaport Faculty of Medicine, which has generated some noble prizes in the last decades.

As in the former years, the principal aim of the meeting was to enable a fruitful international scientific exchange on Gaucher-related issues. The opportunity for presenting unpublished scientific data as well as free discussion is a central premise of the Group and was taken to present the forefront of basic research and clinical advances in Gaucher disease. So, 270 people from more than 40 different countries around the whole world attended.

The scientific part of the meeting showed some 100 presentations and we did like the embedding of most posters into the coffee breaks and placing them directly along the major pathways within the hotel. Some questions were further elucidated during our scientific talks, e.g. the pathophysiology of the disease and new therapeutic options. Nevertheless, a focus was also set around long-term complications of the disease, e.g. plasma cell abnormalities, bone complications or chronic fatigue and we also learnt our lessons on the still hard-to-catch nature of the neurological damage associated with the disease. It occurs that type III is still very hard to improve. No progress has been made on erecting a comprehensive disease registry that allows drawing data for analysis on huge cohorts from different backgrounds, but we still work on it.

During the business meeting Tim Cox and Carla Hollak, who had stepped back in their executive board functions, were thanked for their continuous services to the EWGGD. Hanna Rosenbaum, host of this meeting, and Nadia Belmatoug, who had organized the Paris meeting in 2012, joined the committee. Prof. Elena Lukina, hematologist from Moscow, was elected to host the next, the 12th EWGGD workshop in Moscow at the end of June 2016. Alternate options had been Stockholm, Sweden, Zaragossa in Spain and Prague, Czech Republic.

We truly feel that the 2014 meeting helped to continue the tradition of good scientific quality and was successful to bring enthusiasm to young physicians, researchers and health carers from the whole world in dedicating themselves to patients with this disease.

With deep gratitude we would like to thank Hanna Rosenbaum for her diligent and empathic job. Hopefully meetings like this might contribute to a more peaceful world and it is astonishing that we were blessed with those serene days which, just shortly later, transitioned to frank political atrocities.

The role of the EGA is well-established. We were privileged to join in the celebration of the 20th anniversary of EGA during the 11th EWGGD held in Haifa and express our admiration for what has already been achieved and our wishes for a successful future.

The success of the meeting has inspired us to forward this association and help increase knowledge on Gaucher disease and thereby improve our patients' lives. We are sure everybody will like this informative brochure very much.

Yours very sincerely,

Prof. Stephan vom Dahl,
Chairman EWGGD

Dr. Helen Michelakakis,
Vice Chairman EWGGD

European Gaucher Alliance | Evesham House Business Centre
48-52 Silver Street | Dursley | Gloucestershire | GL11 4ND | UK

Jeremy Manuel O.B.E.
Chairman
Tel & Fax: +44 (0)1453 549231
Email: jeremy@eurogaucher.org
Website: www.eurogaucher.org

Dear friends

On behalf of the members of the EGA, I am very pleased to be bringing to you this publication of the proceedings of the 11th European Working Group on Gaucher Disease. It has become our tradition to publish the proceedings and on this occasion they will be available both electronically and in hard copy.

The EGA undertakes this task not just as a record of what was presented but in the quest of disseminating the information contained in the presentations as widely as possible. We take the view that it is vitally important, and clearly in the best interests of Gaucher patients throughout the world, that the presentations detailing research, discoveries and clinical practices presented at the EWGGD are shared with all those who are interested in the study or treatment of Gaucher disease. Through this publication a reader may be re-visiting a presentation that was heard or reading material for the very first time, but either way I am sure that written within these pages readers will find useful information and novel ideas for treating patients and stimulation for new developments.

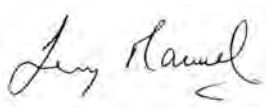
As many will know, the EGA celebrated its 20th Anniversary in Haifa. Before the workshop we held our own biennial meeting. We had 44 attendees from 31 different countries and spent two days sharing experiences, learning of the challenges facing Gaucher patients around the world and developing programmes for our mutual support. Seeing ERT in production with our visit to the Protalix plant was a highlight for many and the opportunity to hear from and question Senior executives from both Genzyme and Shire was extremely valuable.

On behalf of the EGA I would like to thank the EWGGD Chairman Stephan vom Dahl and Vice-Chairman Helen Michelakakis as well as local host Prof Hanna Rosenbaum for their tremendous support in facilitating, with such generosity, the attendance of the EGA representatives at the EWGGD. It was through their efforts and of course the corporate sponsors that the EGA members could participate as fully as they did.

As many will know we launched our EGA film at the EWGGD and this may be viewed at www.eurogaucher.org in the 'downloads' area.

Finally my thanks to Dr Efstathia Chronopoulou and Guilia Massaro who had the task of writing up the various talks and to our very own Jo Cook who assembled this publication and arranged for it to be printed and made available to you.

With very best wishes



Jeremy Manuel OBE
Chairman, European Gaucher Alliance

Index of Presentations in order of delivery

• **The genetic history of the Jewish people**
Karl Skorecki, Israel 10

• **Impact of long term ERT in child, the Spanish experience**
Marcio Andrade, Spain 11

• **Spinal deformities in Norrbottnian type 3 Gaucher disease: prevalence, possible etiology, and management options**
Ehud Lebel, Israel 12

• **Chronic neuronopathic Gaucher disease: outcomes, morbidity in the Mainz cohort**
Eugen Mengel, Germany 13

• **The impact of ERT on immunity in Gaucher disease**
Ozlem Goker-Alpan, USA 13

• **Induced pluripotent stem cell models of Gaucher disease provide new insights into disease pathogenesis and therapy**
Ellen Sidransky, USA 14

• **Aberrant differentiation and impaired lysosomal functions revealed by Gaucher-specific induced pluripotent stem cells (iPSC)**
Ricardo Feldman, USA 15

• **A role for GBA2 in neuropathology in Niemann-Pick type C**
Hans Aerts, Netherlands 15

• **System-wide involvement in Gaucher disease: role of GBA2 and downstream lipids**
Pramod Mistry, USA 16

• **RIPK3 as a potential therapeutic target for Gaucher disease**
Tony Futerman, Israel 16

• **Glycolipid abnormalities in Gaucher disease: is there more to learn?**
Hans Aerts, Netherlands 17

• **The prevalence of Gaucher in idiopathic splenomegaly is 1.50: follow-up of a nationwide screening study in 200 patients**
Stephan vom Dahl, Germany 18

• **High prevalence of small fibre neuropathy in type 1 Gaucher disease**
Annalisa Sechi, Italy 18

• **Whole-body MRI iron level measurement in Gaucher disease**
Martine Regenboog, Netherlands 19

• **Prevalence of autoantibodies in the course of Gaucher disease type 1**
Christine Serratrice, France 19

(continued overleaf)

• **An unsuspected dyserythropoiesis in Gaucher disease**
Caroline LeVan Kim, France 20

• **Taliglucerase alfa in adult patients with Gaucher disease who were previously treated with imiglucerase: 36-month safety and efficacy results**
Ari Zimran, Israel 20

• **Platelet count and chitotriosidase activity in patients with type 1 Gaucher disease who were switched from imiglucerase to velaglucerase alfa**
Deborah Elstein, Israel 21

• **Seven-year follow-up in a phase I/II trial and open-label extension study for velaglucerase alfa in treatment-naïve adults with type 1 Gaucher disease**
Deborah Elstein, Israel 21

• **The UPR-associated transcription factor CHOP upregulates expression of the GBA gene**
Gali Maor, Israel 22

• **Taliglucerase alfa 36-month clinical safety and efficacy in treatment-naïve patients**
Ari Zimran, Israel 23

• **A phase 3, randomized, double blind, placebo controlled, multi center study to investigate the efficacy and safety of eliglustat in adults with Gaucher disease type 1: 18-month results**
Hagit Baris, Israel 23

• **A randomized, controlled, open-label non-inferiority study comparing eliglustat to imiglucerase in Gaucher disease type 1 patients stabilized on enzyme replacement therapy: 24-month results**
Judith Peterschmitt, USA 24

• **Cost-effectiveness studies in Gaucher and Fabry disease**
Carla Hollak, Netherlands 25

• **Novel treatment for Gaucher disease - oral administration of plant cells expressing GCD: Phase 1 study results and Phase 2a program**
Ari Zimran, Israel 25

• **Identification of microRNAs that modulate glucocerebrosidase activity in Gaucher disease cells**
Marina Siebert, USA 26

• **Gaucher disease and parkinsonism: longitudinal clinical characterization and prognosis**
Grisel Lopez, USA 27

• **Comparison of Parkinson risk in Ashkenazi Jewish patients with Gaucher disease and GBA heterozygotes**
Tama Dinur, Israel 27

• **Disruption of monoamine metabolism in lysosomal storage disorders: a mechanistic link to Parkinson's disease?**
Simon Heales, UK 29

• **The connection between ERAD, UPR, Gaucher disease and Parkinson's disease**
Mia Horowitz, Israel 29

• **Visualization of active glucocerebrosidases in rodent brain with high spatial resolution by fluorescent activity based probes**
Daniela Herrera Moro Chao, Netherlands 30

• **The effect of protease inhibition on lysosomal glucocerebrosidase**
Rolf Boot, Netherlands 30

• **Approach to diagnosis and treatment of osteoporosis-state of the art**
Sofia Ish-Shalom, Israel 31

• **Bone assessment in Spanish type 1 Gaucher disease patients: The ZAGAL project**
Marcio Andrade, Spain 32

• **Changes in peripheral blood osteoclast cultures in relation to features of bone disease in adult GD1 patients**
Derralynn Hughes, UK 32

• **Limits on use of health economic assessments for rare diseases**
Jonathan Roos, UK 33

• **The legal imperative for treating rare disorders**
Hanna Hyry, UK 33

Index by Speakers

Aerts Hans 15,17

Andrade Marcio 11,32

Baris Hagit 23

Boot Rolf 30

Dinur Tama 27

Elstein Deborah 21

Feldman Ricardo 15

Futerman Tony 16

Goker-Alpan Ozlem 13

Heales Simon 29

Herrera Moro Chao Daniela 30

Hollak Carla 25

Horowitz Mia 29

Hughes Derralynn 32

Hyry Hanna 33

Ish-Shalom Sofia 31

Lebel Ehud 12

LeVan Kim Caroline 20

Lopez Grisel 27

Maor Gali 22

Mengel Eugen 13

Mistry Pramod 16

Peterschmitt Judith 24

Regenboog Martine 19

Roos Jonathan 33

Sechi Annalisa 18

Serratrice Christine 19

Sidransky Ellen 14

Siebert Marina 26

Skorecki Karl 10

vom Dahl Stephan 18

Zimran Ari 20,23,25

Our Thanks Go To…

The European Gaucher Alliance (EGA) is extremely grateful to Dr Efstathia Chronopoulou and Ms Giulia Massaro who attended the EWGGD in Haifa as guests of the EGA to write the summaries in this supplement, so that our members, families, friends and those interested in Gaucher disease not able to attend this workshop could be kept up to date on what is happening in the field.

Dr Efstathia Chronopoulou is a Paediatric Metabolic Consultant at the Bristol Royal Hospital for Sick Children (UK)and works for the Regional Southwest Paediatric Metabolic Service. She studied medicine in Cambridge and specialised in Paediatrics and Paediatric Metabolic Medicine at the Evelina and Great Ormond Street Children's Hospitals in London. She has an interest in Lysosomal Storage Disorders, especially Gaucher and Fabry disease. She is involved in clinical trials in the field of Paediatric Inherited Metabolic Disease.

Ms Giulia Massaro undertook her higher educations in Italy. Following her undergraduate studies in Molecular Biology at the University of Padova and her postgraduate degree at the University of Trieste, she focused on molecular mechanisms of neurodegenerative diseases at the Molecular Pathology Department at the International Centre for Genetic Engineering and Biotechnology, Trieste. She is currently a PhD student at UCL School of Pharmacy (UK), working on gene therapy for neuropathic Gaucher Disease.

THE GENETIC HISTORY OF THE JEWISH PEOPLE

Skorecki, K. (Israel)

Dr Karl Skorecki opened the meeting with a fascinating presentation on the use of 'genomic science' to study anthropology, demographic history and genealogy. This presentation describes the history of Jewish people through their DNA footprint that put together with sciences such as archaeology and linguistics complete the puzzle.

What can we learn about human history from DNA? What defines Jewish people?

DNA markers provide evidence that all humans are part of one extended family. Many members of worldwide Diaspora Jewish communities show genomic evidence of shared ancestry as a branch of the extended family with Near East origins.

During DNA replication, errors occur that lead to living diversity with illness as a price to pay. DNA markers are silent errors that account for the largest proportion of DNA replication errors at around 95%. A genetic marker is a variation in the nucleotide sequence of the DNA, known as a mutation. Mutations which occur within genes -- a part of the DNA which codes for a protein -- may cause a malfunction or disease and its subsequent spread or loss is modified by evolutionary selective factors succeeding generations. However, mutations found in so-called "non-coding regions" of the DNA tend are influenced principally by demographic history.

These DNA markers are called SNPs (Single Nucleotide Polymorphism), i.e. single letter copying errors and STRs (Simple Tandem Repeat) i.e. variation in repetitive letters. There are approximately 20 million SNPs in total, so long as a threshold of > 1% in the human population is used, and of course there are many more "private" or "family specific" SNPs that have not yet, or will not reach or exceed this threshold.. Demographic factors affect genome wide allele frequencies. We can think of the SNPs as branches and the STRs as twigs of a genealogical tree.

SNPs and STRs on the Y chromosome are used to trace the paternal lineage, while mitochondrial ones are used to trace the maternal lineage. Ancestry is defined by sharing the same set of markers for a given DNA region.

Since the Y chromosome consists almost entirely of non-coding DNA (except for the genes determining maleness), it would tend to accumulate mutations. Since it is passed from father to son without recombination, the genetic information on a Y chromosome of a man living today is basically the same as that of his ancient male ancestors, except for the rare mutations that occur along the hereditary line.

A combination of these neutral mutations, known as a haplotype, can serve as a genetic signature of a man's male ancestry. Maternal genealogies are also being studied by means of the m-DNA (mitrocondrial DNA), which is inherited only from the mother. Mitochondrial DNA sequence relations reconstruct the maternal genealogic tree. Four founding mothers of Near East origin account for 40% of Ashkenazi Jews

Dr. Skorecki considered a hypothesis: if the Cohanim are descendants of one man, they should have a common set of genetic markers -- a common haplotype -- that of their common ancestor.

A study was undertaken to test the hypothesis. If there were a common ancestor, the Cohanim should have common genetic markers at a higher frequency than the general Jewish population.

In the first study, as reported in the prestigious British science journal, *Nature* (January 2, 1997), 188 Jewish males were asked to contribute some cheek cells from which their DNA was extracted for study. Participants from Israel, England and North America were asked to identify whether they were a Cohen, Levi or Israelite, and to identify their family background. The results of the analysis of the Y chromosome markers of the Cohanim and non-Cohanim were indeed significantly clustered in a follow up study, Dr. Skorecki and associates gathered more DNA samples and expanded their selection of Y chromosome markers. Solidifying their hypothesis of, they found that a particular array of six chromosomal markers was found in 97 of the 106 Cohens tested. This collection of markers has come to be known as the Cohen Modal Hapoltype (CMH) -- the standard genetic signature of the Jewish priestly family. The chances of these findings happening at random are greater than one in 10,000. In still further studies a limited number of additional lineages were found, with the lineage bearing the CMH being most common and dating back some 100 generations.

Wider genetic studies of diverse present day Jewish communities show a remarkable genetic cohesiveness. Jews from Iran, Iraq, North Africa and European Ashkenazim show remarkable clustering with each other and with other Semitic groups, with a Levant geographic origin.

The most recent genetic research has clearly refuted the libel that the vast majority of Ashkenazi Jews have no relation to the ancient Hebrews, but are descendants of the Kazar tribe -- a pre-10th century Turko-Asian empire which reportedly converted en masse to Judaism. Researchers compared the DNA signature of the Ashkenazi Jews against those of many contemporary populations that might be candidate descendants of the Kazar kingdom – and found no correspondence.

In subsequent publications the researchers included an unexpected finding. Those Jews in the study who identified themselves as Levites did not show a set of markers as well clustered as did the Cohanim. According to tradition, the Levites should also show a genetic signature from a common paternal patrilineal ancestor. The researchers are now focusing effort on the study of Levites' genetic make up to learn more about their history in the Diaspora.

Using the CMH as a DNA signature of the ancient Hebrews, researchers are pursuing a hunt for DNA sequence markers and patterns in several global population and communities that may point to male or female or biparental Jewish heritage.

Y chromosome and mtDNA results as well as genome-wide analyses also tend to favour founder and bottleneck effects often followed by population expansion bringing certain DNA markers and also certain disease causing or pre-disposing genetic variants to high levels. Three bottlenecks can be envisaged: 1) 3000ybp ago in the Near East, 2) 2000ybp ago prrio to the Ashkenazi founder event(s), and 3) during the past 1000 years Ashkenazi. Ashkenazi Jews were founded from a small group that underwent massive numerical expansion through endogamy.

IMPACT OF LONG TERM ERT IN CHILD, THE SPANISH EXPERIENCE

Andrade, M., Alfonso, P., Irún, P.,Dalmau, J., Barbera, J.L., Cano, H., Fernandez-Galán, M.A., Franco, R., Gracia, I., Ibañez, A., Lendines, F., Martin-Hernández, E., Pérez del Soto, A., Sancho-Val, I., Sanjurjo, P., Pocovi, M. and Giraldo, P. (Spain)

Dr Andrade presented the Spanish experience of treating children with Gaucher disease for the last 20 years.

We know that ERT modifies the natural history of GD. 49% of GD1 patients are diagnosed before the age of 10 years. They have more severe disease and major problems such as growth retardation, bone crisis, bone deformity and pain and the consequences of splenectomy. Bone disease is the most frequent complication.

There are 376 patients registered in the in the Spanish Registry of Gaucher disease, 92 of whom are from Portugal. From the 376 patients registered, 79 were diagnosed during childhood. 26 had GD2, 13 GD3 and 40 GD1. Only the 53 GD1 and GD3 patients were included in the analysis. Mean age at diagnosis was 5.8 years and 8.5 years was the mean age at the start of treatment. The L444P/L444P was the most frequent genetic mutation in the GD3 and the N370S/L444P in the GD1 patients. 3 patients were splenectomised before the diagnosis. 60% had a bone marrow or a liver biopsy to make the diagnosis. 85% received ERT and 21 of them were on ERT for more than 15 years. Three quarters of the treated patients received Imiglucerase and the remaining received Velaglucerase and Taliglucerase (6.7 and 8.9% respectively).

50% of the patients had bone disease and 25% had the Erlenmeyer deformity at baseline.

At follow-up, 4 female patients experienced moderate to severe infusion reactions with IgG anti-imiglucerase antibodies detected on serum,2 of them abandoned treatment. Haematological parameters, including clotting abnormalities, haemoglobin and platelets normalised on treatment. Liver and spleen size improved. Chitotriosidase also improved.

Less than 25% had bone disease and 3 had bone crisis as opposed to 7 at baseline. 1 patient had a joint replacement. 2 patients had osteopenia at 2 years of treatment. 1 patient developed early diabetes and one thyroiditis.

In summary, GD related complications in children on ERT are lower than in adults (22 versus 70%). Endocrine complications are rare and not related to ERT. The N370S heterozygote patient shows growth retardation more frequently than patients who carry other mutations.

SPINAL DEFORMITIES IN NORRBOTTNIAN TYPE 3 GAUCHER DISEASES: PREVALENCE, POSSIBLE AETIOLOGY, AND MANAGEMENT OPTIONS

Lebel, E., Kämpe Björkvall, C., Nilsson, M., Klimkowska, M., Myhr-Eriksson, K., Elstein, D., Svenningsson, P. and Machaczka, M. (Israel/Sweden)

Dr Lebel presented this paper which is a collaborative study between Northern Sweden and Israel.

The L444P homozygous GD 3 patients follow a similar disease path with slow neurological progression and a typical spinal deformity. Osteoporosis-related vertebral fractures were thought to be the cause of this problem. As these patients now live longer on treatment, this study was designed to look further into the cause and better management of the spinal deformity.

In this study, 15 patients were included, 8 were females and 7 males. Age range was between 4 and 61 years. 9 out of the 15 were splenectomised with a median age of 7 years at splenectomy. 7 patients had epilepsy with 24 years the median age of onset. Older patients had dystonia and children above the age of 3 had oculomotor apraxia. 2 patients received BMT and those on ERT were treated for a mean period of 17 years.

Looking at the skeletal abnormalities, they have no long bone deformities and osteonecrosis is rare, possibly due to long term ERT. However, spinal deformities are prevalent in all patients. These consist of a slowly developing kyphoscoliosis and a compensatory hyper lordosis in the cervical and lumbar regions of the spinal column. This leads to trunk height loss as well as balance and head positioning problems. Spinal deformity adversely affected independence. 3 patients had spinal surgery that halted spinal deterioration.

All 15 patients had sagittal spine deformity, the children included. A scoliosis was detected in only the older patients. Very importantly, the severity of the spinal deformity parallels the neurological deterioration.

As a result of the spinal deterioration, patients develop respiratory insufficiency, diminished horizontal vision and difficulty in swallowing while being fully mobile independently.

As there is no vertebral compression fracture detected, the research group hypothesise that the spinal deformity has a neurological aetiology. Therefore, spinal orthodesis or fusion can potentially halt disease progression. This is the preferred intervention in non Gaucher disease neurological spinal problems. The general good condition of the patients allows intervention before the kyphosis becomes extreme.

In summary, spinal deformity is one feature of Norrbottnian GD 3 that is possibly neurogenic in origin. Management requires annual monitoring with a view to intervention as indicated. It may also be present in other GD 3 patients.

CHRONIC NEURONOPATHIC GAUCHER DISEASE: OUTCOMES, MORBIDITY AND MORTALITY IN THE MAINZ COHORT

Mengel, E., Arndt, J., Brixius-Huth, M., Bremova, T., Naumann, S. and Reinke, J (Germany)

Dr Mengel presented the natural history data for the German GD 3 cohort. This is very important in the light of emerging therapeutic options in order to look at outcomes and study endpoints.

25 patients were followed –up aged 2 to 42 years from 1991 -2014. Follow-up was 1-18 years. In the adult onset group, patients were between 22-42 years and the diagnosis made between 20-32 years. They all had mild visceral disease and were of German origin. The presenting features were oculomotor apraxia and seizures. The genotypes were L444P/L444P and L444P/other.

9 patients died during the study period, 6 due to neurological and 3 due to visceral complications. There were 3 deaths in early life.

One patient with protein-losing enteropathy and thickening of the small bowel wall surrounded by mesenteric lymph nodes was treated with ERT and eliglustat. 2 patients developed severe kyphoscoliosis that needed surgery.

Myoclonic encephalopathy was associated with neurological decline.

Modified severity scoring tests are based on motor function as patients believe it to be the most important parameter that allows independent life. Motor function includes ataxia of gait, cerebellar tremor, pyramidal and extrapyramidal signs.

Abnormal saccadic eye movements were detected in all patients. However, age does not correlate with velocity making this a less useful potential study endpoint.

The researchers conclude that mortality and morbidity in GD 3 is due to neurological and visceral complications which may not respond to ERT. Motor function tests should be used as useful endpoints in clinical trials.

THE IMPACT OF ERT ON IMMUNITY IN GAUCHER DISEASE

Goker-Alpan, O., Komlodi-Pazstor,E., Limgala, R., Martin, C., Hebert, A., Brown, M., Plassmeyer, M., Ryherd, M., Austin, L. and Alpan, O. (USA)

In this talk, Dr Goker-Alpan discussed the immune system abnormalities in GD patients. These may affect both arms of the immune system, which are the antibodies and the cell that fight infection. This is also shown in animal models.

This was assessed in a study of 27 patients between 5 and 64 years of age over 4 years. 19 females and 8 males were included. 5 treatment naive patients were also included.

Special techniques used to look at lymphocyte subsets and function, were used to analyse the samples taken before and after ERT and at indicated intervals.

The results showed that 1/3 of the patients had elevated immature B cells. After treatment this normalised showing improved B cell maturation. Therefore, ERT may correct the B-cell maturation defect in GD1 patients.

Similar alterations were also found in the T-cells and dendritic cells of the immune system.

These alterations correlate with the time of commencement of ERT and/or interruptions. These immune changes may be reversible and amenable to treatment. These alterations may play a role in the development of haematological malignancies seen later on in life in GD patients.

INDUCED PLURIPOTENT STEM CELL MODELS OF GAUCHER DISEASE PROVIDE NEW INSIGHTS INTO DISEASE PATHOGENESIS AND THERAPY

Sidransky, E. (USA)

New models to study macrophages, the cells most affected in GD, and the association between GD and PD is critical in order to evaluate clinical variation in patients and to develop new treatment strategies. Current models do not reproduce the cellular changes occurring in patients. Both animal models (knock-out, conditional knock-out, knock-in) and *in vitro* models (cultured fibroblasts) have many limitations and the understanding of the pathogenesis is challenging. Studies on genotype and phenotype are useful, but often there is not good correlation between the two. In fact, clinically different patients can have the same genotype and clinically similar patients have many different genotypes.

The researchers generated an induced-pluripotent stem cell (IPSC) model deriving from GD fibroblasts. The iPSCs were then used to make human macrophages. Glucocerebrosidase (GCase) activity is reduced and glycolipid storage is increased in these GD cells. Moreover, chemotaxis and autophagy is compromised.

IPSC-macrophages were used in order to evaluate the efficacy of a novel chemical chaperone identified by high throughput screening of more than 250,000 compounds. The molecule enhanced the translocation of glucocerebrosidase to the lysosome. In addition, adding lipids to these cultured cells resulted in the abnormal lipid storage seen in GD. This storage was reversed with the chemical chaperone and the enzyme activity was restored. Also the impaired chemotaxis was corrected.

Since mutations in the GBA gene have been found in Parkinson's patients, the study used the IPSC model in order to further understand the pathogenesis of PD and the effect of this new enzyme-enhancing therapy. Dopaminergic neurons were derived from IPSCs and the link between increased levels of α-synuclein and the deficiency in GCase was studied. After the treatment with the chemical chaperone, neurons restored their GCase activity and the amount of α-synuclein was reduced.

This study demonstrated the utility of this novel GD macrophage model and proposed new insights regarding the correlation between GBA mutations and the accumulation of α-synuclein in PD. The goal is to ultimately identify new drugs that could be helpful for both patients with GD and Parkinson disease.

ABERRANT DIFFERENTIATION AND IMPAIRED LYSOSOMAL FUNCTIONS REVEALED BY GAUCHER-SPECIFIC INDUCED PLURIPOTENT STEM CELLS (iPSC)

Feldman, R.A., Panicker, L.M., Sgambato, J.A., Awad, O. and Miller, D. (USA)

To model Gaucher disease (GD) we generated Induced pluripotent stem cells (iPSC) from fibroblasts of patients with types 1, 2 and 3 GD, and these cells were differentiated to cell types affected by GD, including macrophages, hematopoietic progenitors and neurons. GD macrophages had a striking defect in their ability to clear phagocytosed RBC, recapitulating a characteristic hallmark of GD, and the extent of this effect correlated with severity of the mutation. The mutant macrophages were constitutively activated and produced elevated levels of TNF-alpha, IL-1beta and IL-6, which may increase the risk of developing multiple myeloma. The abnormal phenotypes we observed were reversed by incubation with recombinant glucocerebrosidase (rGCase) or small molecules, to an extent that reflected their known clinical efficacies. Ambroxol was the most effective of the chaperones tested. Directed differentiation of GD iPSC to lineage-restricted hematopoietic cells (erythroid and myeloid lineages) showed that GCase deficiency caused developmental defects, with decreased differentiation of erythroid cells and aberrant myeloid differentiation, including the appearance of abnormal macrophage-like cells. These abnormalities reflect the cytopenias seen in GD patients, and suggest that Gaucher macrophages may be derived from abnormal myeloid progenitors. rGCase reverted these abnormal phenotype and prevented the appearance of aberrant macrophage-like cells. Directed differentiation of GD iPSC to neurons showed that GCase mutations caused widespread lysosomal depletion, and a block in autophagic flux due to defective clearance of autophagosomes by the lysosomes. These effects were seen in types 2 and 3 but not type 1 neurons, and were reversed by rGCase. In sum, GCase mutations cause a block in the transport of cargo to the lysosome and interfere with lysosomal clearing functions, which is particularly detrimental in neurons. In addition GCase deficiency causes intrinsic developmental defects by interfering with differentiation and maturation in different cell types. In sum, patient-derived iPSC provide a very relevant experimental system to elucidate the molecular mechanisms that underlie the pathophysiology of GD, and will have an impact on therapeutic development.

A ROLE OF GBA2 IN NEUROPATHOLOGY IN NIEMANN-PICK TYPE C

Aerts, J.M.F.G., Marques, A.R.A., Aten, J., Ottenhoff, R., van Roomen, C.P.A.A., Claessen, N., Mirzaian, M., Boot, R.G., Yildiz, Y. and Overkleeft, H.S. (Netherlands)

Non-lysosomal glucocerebrosidase (GBA2) is a membrane protein that degrades glucosylceramide (GlcCer) to ceramide and glucose and can also add a cholesterol molecule to □-D-glucoside. When the lysosomal GBA1 enzyme is deficient, the hyperactivity of GBA2 compensates the impaired lysosomal GlcCer degradation. However, the enhanced GBA2 activity could lead to toxic accumulation of ceramide in the cytosol. N-(5'-adamantene-1'-yl-methoxy)-pentyl-1-deoxynojirimycin (AMP-DNM) is a potent irreversible GBA2 inhibitor.

The neurological symptoms of Niemann-Pick disease are correlated with the accumulation of primary sphingolipids in the lysosomal compartment. In particular, an increase of GlcCer has been reported, suggesting that GBA1 activity is compromised in NPC fibroblast.

This study used Niemann-Pick mouse (*Npc1*$^{-/-}$) as a model to suggest the implication of GBA2 in neuropathology in NPC type C disease. The activity of GBA2 was increased in NPC mice and the enzyme has been found predominantly within Purkinje cells. The pharmacological inhibition of GBA2 in *Npc1*$^{-/-}$ mice increased survival and delayed the onset of neurological symptoms. Even if the inhibition of GBA2 activity rescued the abnormal phenotype, administration of AMP-DNM should not be considered a cure because it does not correlate with lysosomal GBA1 defects.

Double knock-out mice where generated (*Npc1*$^{-/-}$/*Gba2*$^{-/-}$). The depletion of GBA2 increased life span and delayed abnormal motor coordination.

SYSTEM-WIDE INVOLVEMENT IN GAUCHER DISEASE: ROLE OF GBA2 AND DOWNSTREAM BIOACTIVE LIPIDS

Mistry, P.K. (USA)

In GD patients, the risk of developing other pathologies likes myeloma, haematological cancer, melanoma, osteoporosis, avascular osteonecrosis, pulmonary hypertension and Parkinson's Disease, is increased but not always correlated with GD severity.

The aim of this study was to investigate the implication of downstream lipids in GD and suggest the use of sphingosine as biomarkers. The accumulation of sphingolipids occurs primarily in the lysosomal compartment; consequently, the lipids are transferred outside the lysosome. This mechanism could potentially involve the non-lysosomal glucocerebrosidase enzyme (GBA2).

Conditional *Gba* knock-out GD1 mice (*Mx1-Cre*+) were used to show that the immune system is compromised in GD. In fact, mice developed hypercytokinemia, B-cell recruitment was disrupted, antigen presentation was defective, T-cells were impaired in thymus and cytopenia has been reported as a consequence of splenomegaly. Moreover, the earlier temporal elevation of glucosylceramide and glucosylsphingosine (LysoGL1) was detected.

An increase in LysoGL1 levels leaded to inhibition of osteoblast viability, but did not affect osteoclasts. The correlation between severity of GD phenotype and osteopenia has been proved.

Depletion of the *Gba2* gene rescued the phenotype and attenuated hypercytokinemia in GD1 mice.

RIPK3 AS A POTENTIAL THERAPEUTIC TARGET FOR GAUCHER DISEASE

Futerman, T. (Israel)

Professor Futerman gave us a fascinating talk on his latest research that can help identify novel therapeutic targets for Gaucher disease.

Glucosylceramide (GlcCer) and glucosylsphingosine accumulation in the brain leads to massive neuronal loss in the neuronapathic GD patients and mouse models. Type-1 Gaucher disease does not involve the brain. Type 2 — the most severe form — causes extensive brain damage and death before two years of age, while Type 3 is a more progressive form of the disease that affects the brain, with patients often living into their early teens and adulthood.

Despite research in the field of Gaucher disease in the last few decades, there

remain many open issues: 1) the molecular details of inflammation 2) the molecular explanation for the clinical variability 3) the mechanism of the neuro inflammation and the regional selectivity in affected brain areas. Answers to the first two questions could potentially lead to novel treatment regimens while the answer to the third question could determine the mechanistic link to Parkinson's disease.

The Parkinson's connection and its prevalence in other LSDs, could either be explained by the misfolded GBA enzyme or by a defect in lysosomal function. Evidence to date to support either is unsatisfactory.

Dr Futerman's research group used conduritol-B epoxide (CBE), an inhibitor of GlcCer breakdown to induce neuronal Gaucher disease in mice CBE is an active site inhibitor for the enzyme, the dose and timing of which can be manipulated to induce different types of GD.

Fluorescent labelling of layer V of the cerebral cortex shows considerable neuronal cell loss in the CBE-treated mice with a subsequent stop in neuronal degeneration when CBE is removed. Despite glucosylceramide (GlcCer) accumulating in all areas of the brain, there is selective neuronal vulnerability possibly due to intrinsic properties of the neurons. There are no significant changes in any other lipids. There are changes in lysosome distribution with GlcCer accumulation that lead to misplaced lysosomes and cell death.

Neuronal cell death is non-apoptotic and caspase-independent. There is increase in chemokines and cytokines particularly TNFa with no clinical response to anti-inflammatory drugs.

Dr Futerman's group wanted to find out exactly what causes such a massive loss of nerve cells in Types 2 and 3 Gaucher disease. A biochemical pathway, of which a protein called RIP3 is a key player, is involved in triggering the cell death and inflammatory processes that can have severe consequences in a number of diseases. In order to investigate whether this could also be one of the missing links in the understanding of the chain of molecular events leading to brain inflammation and nerve cell death in Gaucher disease, they induced Gaucher disease in mice possessing the RIP3 protein, as well as in mice lacking RIP3. The scientists showed that the mice lacking the RIP3 protein demonstrated not only a significant improvement in motor coordination and brain pathology, but also improved liver and spleen function. Their lifespan was also remarkably increased, from approximately 35 days to more than 170 days.

The researchers hypothesize that this protein is involved in cell death through a process of necroptosis. This pathway is also important in other neurological diseases. It is a promising target for the development of inhibitors as a novel therapy for Gaucher disease.

GLYCOLIPID ABNORMALITEIS IN GAUCHER DISEASE: IS THERE MORE TO LEARN?

Aerts, J.M.F.G. (Netherlands)

The presentation intends to present food for thought about the complex molecular abnormalities associated with deficiency of lysosomal glucocerebrosidase (GBA1), the primary defect in Gaucher disease patients. Glucocerebrosidase (GBA1) is at first look a simple degrading hydrolase. It cleaves the substrate glucosylceramide via a two-step reaction: first, glucose is removed from glucosylceramide; then, a water molecule enters the catalytic pocket and free glucose is realised. This reaction mechanism has offered the possibility to design so-called activity based probes allowing ultrasensitive visualisation of active GBA1 molecules in living cells and animals. Closer inspection reveals that the function of GBA1 is far more intricate. In fact, it can also catalyse the removal of xylose from hydrophobic structures. Of note for the physiological relevance of this, excessive deposition in the heart of glycosaminoglycans, structures containing ß-linked xyloside, is reported for specific GD patients. GBA1 is also promiscuous in degrading glucosidic substrates. Recent own unpublished work has revealed that cholesterol-glucoside, another glycolipid, also accumulates in spleen, bone marrow and plasma of GD model mice when GBA1 activity is deficient. As observed by us, cholesterol-glucoside is an excellent substrate for GBA1 in test tube assays. Enzymes like GBA1 can intrinsically also transglycosylate: donating the glucose removed from glucosylceramide to another acceptor compound. In order to identify potential acceptors

in the transglycosylation site, researchers have developed a new technology based on chemical compounds. These are sugar mimics that allowed labelling the newly formed metabolites formed by transglycosylation. In this way it was detected that cholesterol-glucoside is not only degraded by GBA1 but also formed by transglycosylation by the same enzyme.

The food for thought of the lecture is to point out that restoration of normal enzyme (that means enzyme replacement therapy) resolves *a priori* the multiple known, and possibly yet unknown, biochemical abnormalities related to deficiency of the enzyme.

THE PREVALENCE OF GAUCHER IN IDIOPATHIC SPLENOMEGALY IS 1.50: FOLLOW-UP OF A NATIONWIDE SCREENING STUDY IN 200 PATIENTS

vom Dahl, S., Bange, M., Merkel, M., Voßbeck, J., Mengel, E., Herrmann, A., Donner, M., Santosa, D. and Häussinger, D. (Germany)

This study aimed to determine the prevalence of GD in patients with idiopathic splenomegaly in a German population from 2008 to 2012. Follow-up with clinical data from the patients was recovered from nine German centres.

The main characteristics of splenomegaly are: palpable spleen, volume >750 ml, depth >4cm, width >7cm, length >11cm. In the presence of idiopathic splenomegaly, the patient should be checked directly for GD.

4 out of 200 patients diagnosed with idiopathic splenomegaly had GD (GBA and chitotriosidase activity). These patients developed thrombocytopenia and hepatoslenomegaly.

It was suggested that the diagnosis should not rely completely on bone marrow biopsy, as GD cells are hard to find in bone marrow smears.

HIGH PREVALENCE OF SMALL FIBRE NEUROPATHY IN TYPE 1 GAUCHER DISEASE

Sechi, A., Devigili, G., Ciana, G., Deroma, L., Dardis, A., Zampieri, S., Deganuto, M., Cattarossi, S., Pianta, A., De Filippo, M., Lettieri, C., Rinaldo, S., Eleopra, R. and Bembi, B. (Italy)

Persisting pain is a common feature among GD patients, even in those who have been receiving enzyme replacement therapy (ERT) for more than ten years. Even if bone crisis is not reported, the patients still feel pain.

The aim of the study was to investigate the prevalence of neuropathic pain in GD type 1 patients and the possible involvement of small fibre neuropathy (SFN).

25 GD patients were tested with multimodal Quantitative Sensory Testing (QST), Douler Neuropatique questionnaire (DN4), Neuropathic Pain Symptom Inventory (NPSI) and skin biopsy, with quantification of intraepidermal nerve fibres at distal legs and proximal thigh. 12 patients had chronic pain and 20 presented epidermal denervation (13 with no length-dependent pattern). They had a reduced sensitivity to cold and paradoxical sensation.

This study suggested that pain in GD type 1 is caused by not only bone disease but also SFN.

WHOLE-BODY MRI IRON LEVEL MEASUREMENT IN GAUCHER DISEASE

Regenboog, M., Akkerman, E.M., Stoker, J. and Hollak, C.E.M. (Netherlands)

Iron can be found in Gaucher cells and therefore it is possible that depots of iron in the body could be used as a marker of Gaucher disease (GD). Residual disease burden in GD may be related to the occurrence of late complications or associated conditions. With whole-body MRI iron level measurement, we introduce a novel approach to evaluate residual disease activity.

The study protocol is presented and the first results are shown at the meeting.

MRI images are obtained using a 1.5T-scanner. Whole-body T2* measurements are done and R2* values (inversely proportional to T2*, correlation with iron concentration) are reported for each organ studied.

The study will comprise 40 GD patients and matched healthy controls. The first participants show a variety in iron levels, but remarkable differences between GD patients and a healthy control were found.

The first study results suggest that MRI could be an additional tool for screening and identification of the typical locations of iron storage in GD. This could be of use in evaluating residual disease activity. More experiments will be performed in order to collect more data. Also the role of ferritin and hepcidin will be studied in relation to a possible altered iron homeostasis in GD.

PREVALENCE OF AUTOANTIBODIES IN THE COURSE OF GAUCHER DISEASE TYPE 1

Serratrice, C., Bensalah, N., Belmatoug, N., Masseau, A., Rose, C., Kaminsky, P., Lidove, O., Camou, F., Maillot, F., Leguy Seguin, V., Bertrand, N.M., Marie, I., Cabane, J., Alessandrini, M., Bardin, N., Boucraut, J. and Berger, M. (France)

GD patients usually develop hypergammaglobulinemia. Cases of haemolytic anemia and immune thrombocytopenia have also been reported.

This study aimed to establish the presence of autoantibodies and other autoimmune disorders in GD patients.

40 GD patients and 20 healthy controls were studied. The affected patients were treated with enzyme replacement therapy (ERT) and carried the N3705S mutation (47% heterozygotes). The blood samples were tested for antinuclear, anti ENA, antiphospholipids and antigangliosides. The analysis revealed that 50% of GD patients had autoantibodies, 15% antinuclear, 27% antiphospholipids and 12% antigangliosides.

The study was not able to demonstrate the link between the presence of autoantibodies and genotype and splenectomy. There was no evidence of correlation with autoimmune disease, even though the presence of autoantibodies is more frequent in GD patients. The involvement of cytokines has been suggested, but other mechanisms should be analysed.

AN UNEXPECTED DYSERYTHROPOIESIS IN GAUCHER DISEASE

LeVan Kim, C., Reihani, N., Arlet, J.B., Billette de villemeur, T., Belmatoug, N., Colin, Y., Hermine, O. and Franco, M. (France)

Red blood cells (RBC) aggregation and blood viscosity are impaired in Gaucher disease (GD) affected patients. Flow adhesion experiments revealed abnormal dynamic adhesion properties, suggesting that the compromised RBC can contribute to the development of vascular occlusion events.

The study aimed to determine if the erythroid progenitors are directly affected.

A two-step liquid culture from 20ml of blood (20 GD type 1 patients and controls) was used to induce *in vitro* erythropoiesis from circulating progenitors and GBase activity was detected in CD34+ cells. During the first phase of erythropoiesis, a decrease in proliferation has been reported. Moreover, the erythroid terminal differentiation is accelerated in cells derived from GD patients. Subsequently, the effect of GD macrophages on terminal erythropoiesis was studied. Macrophages derived from both patients and controls equally improved erythroid differentiation. This result highlighted that the dysfunction should be within the erythroid lineage of GD affected patients.

In the future, the study will investigate the role of sphingosine and other downstream bioactive lipids on erythropoiesis and RBC properties.

TALIGLUCERASE ALFA IN ADULT PATIENTS WITH GAUCHER DISEASE WHO WERE PREVIOUSLY TREATED WITH IMIGLUCERASE: 36-MONTH SAFETY AND EFFICACY RESULTS

Zimran, A., Pastores, G.M., Shankar, S.P., Petakov, M., Giraldo, P., Rosenbaum, H., Amato, D.J., Szer, J., Chertkoff R. and Brill-Almon, E. (Israel/Ireland/USA/Serbia/Spain/Canada/Australia)

Taliglucerase alfa is a new recombinant protein used in enzyme replacement therapy (ERT) approved for treatment of adults affected by GD type 1.

This study aimed to extend the safety and efficacy data on taliglucerase.

The phase 3 study comprised 9 patients, both male and female, who have been previously treated with imiglucerase for at least two years. An equal dose of taliglucerase has been given. Patients had stable disease parameters and stable enzymatic activity, they had no severe neurological symptoms and were not taking other experimental medications. The study has been conducted for 36 months.

After 9 months of treatment one patient developed an allergic reaction and 18 went to phase 003. At the end of the 36-month period, 14 completed the study. All treatment-related adverse events (nasopharyngitis, headache and pain) were mild/moderate and transient. Only one patient developed severe adverse effects, but it was correlated to a pre-existing pathology.

The efficacy results showed the stabilization of platelet count and haemoglobin concentration, stability of the liver's dimensions and reduction in the liver's dimensions (from 4.6 to 3.7 MN).

(continued overleaf)

PLATELET COUNT AND CHITOTRIOSIDASE ACTIVITY IN PATIENTS WITH TYPE 1 GAUCHER DISEASE WHO WERE SWITCHED FROM IMIGLUCERASE TO VELAGLUCERASE ALFA.

Elstein, D., Zimran, A., Hughes, D.A., Giraldo, P., Charrow, J., Smith, L., Shankar, S.P., Kunes, Y., Wang, N., Dinh, Q., Crombez, E. and Mehta, A. (Israel/UK/Spain/USA)

Patients with type 1 Gaucher disease who switched from imiglucerase to velaglucerase alfa were evaluated in a 12-month trial, TKT034, and extension study HGT-GCB-044; a post hoc, exploratory sub-analysis of TKT034-extension data was performed to specifically evaluate changes in platelet counts and chitotriosidase activity in these patients. 27/30 patients (90%) with normal Baseline platelet counts ($\geq 120 \times 10^9$/L) either were stable ($\leq$20% change) or had improved by >20% at the time of the last assessment. 4/8 (50%) who had below-normal platelet counts before switching normalized at a post-Baseline assessment. 38 patients were studied. 21/22 patients (95%) with Baseline chitotriosidase values $\leq$5000 nmol/mL/h were stable ($\leq$15% change) or had improved (<-15%) at the last assessment. 9/15 (60%) who had Baseline values >5000 saw the measurement decrease to $\leq$5000 nmol/mL/h at a post-Baseline assessment. The conclusion was that platelet counts and chitotriosidase measurements were stable or improved in almost all 38 patients in these studies switched from long-term imiglucerase. The possibility of achieving near-normal values with Gaucher-specific treatment should be explored.

SEVEN-YEAR FOLLOW-UP IN A PHASE I/II TRIAL AND OPEN-LABEL EXTENSION STUDY FOR VELAGLUCERASE ALFA IN TREATMENT-NAIVE ADULTS WITH TYPE 1 GAUCHER DISEASE

Elstein, D., Wang, N., Ogg, C., Crombez, E., Cohn, G.M. and Zimran, A. (Israel/USA)

Dr Elstein presented the results of the 7-year clinical trials using velaglucerase in adult patients with GD 1. Velaglucerase alfa has the wild type sequence of glucocerebrosidase. TKT025 was its first clinical trial in patients with 9 month's duration and TKT025EXT is an extension clinical trial of 80-89 months' cumulative study-drug exposure.

Patients were started on a dose of 60U/kg every other week for the first 15-18 months and gradually reduced to 30U/kg if they met 2 out of the 4 therapeutic goals as described previously. 7 out of the 10 patients were Israelis on home therapy.

Main assessments were safety (i.e., incidence of adverse events, antibody formation) and efficacy measurements (improvements in haemoglobin & platelet counts, and reduction in spleen and liver volumes), plus changes in biomarkers and exploratory bone evaluations (such as bone marrow burden and bone mineral density).

8/10 patients completed the extension study: 2 were withdrawn due to pregnancy.

Safety assessment results showed that no patients discontinued due to adverse events or developed anti-drug antibodies and none required pre-medication at any point. Statistically significant mean changes from pre-TKT025

values were observed in haemoglobin concentration, platelet counts, liver volume, and spleen volume. Long-term therapeutic goals for these variables and bone mineral density were achieved in 8/8 patients. For haemoglobin, platelets, and liver volume, most or all patients with abnormal baseline values achieved normalization or near-normalization, some as early as by 3-6 months of treatment. Bone mineral density Z-scores increased from 24 months in the region of the lumbar spine and from 33 months in the region of the neck of femur.

This was the longest clinical trial for patients with Gaucher disease with robust safety and efficacy data using velaglucerase alfa.

THE UPR-ASSOCIATED TRANSCRIPTION FACTOR CHOP UP REGULATES EXPRESSION OF THE GBA GENE

Maor, G., Braunstein, H. and Horowitz, M. (Israel)

Ms Gali Maor, a Ph D student in the Horowitz lab, gave this fascinating talk about deciphering the pathogenesis of Gaucher disease at the molecular/cellular level that is crucial in designing new therapies.

Inside cells, the endoplasmic reticulum (ER) is the cellular organelle responsible for protein maturation. The proteins are folded in this organelle and are then released and targeted to the cellular compartment they are destined for. However, if the protein formed is faulty, as in the case of mutant glucocerebrosidase, it is not released appropriately and remains trapped inside the ER. As a means to destroy it, the faulty protein is removed from the ER to the cytoplasm, where it undergoes ubiquitination and degradation. Gali Maor has shown that Unfolded Protein Response (UPR) is activated in GD cells and cells carrying GD mutations.

Transcription factors are powerful proteins in the cells that promote reading from any gene and making the protein product its genetic alphabet coded for. Two such factors, namely CHOP and Xbp1, are turned on by the UPR.

In GD, glucocerebrosidase mRNA levels, which are the transcripts that give the instructions for the production of more enzyme, are increased when compared with healthy cells. Very sensitive genetic techniques that are employed in the lab have shown that this genetic material soon to be translated to glucocerebrosidase (GCase) is not only increased in cells from GD patients but also in cells that originated from carriers on GD mutations. The transcription factor CHOP has a binding site on the GBA gene promoter region which is the region that will start off reading of the genetic instructions and GCase enzyme production.

In very elegant experiments, the promoter region of the GBA gene was joined to a gene expressing luciferase and makes it possible to measure the GBA promoter's activity. The results showed that the normal GBA promoter was activated by CHOP but the GBA promoter mutated in its CHOP binding site was not.

Therefore, UPR activation in GD cells and cells carrying different GD mutations causes induction of GBA transcription through the action of the transcription factor CHOP.

TALIGLUCERASE ALFA 36-MONTH CLINICAL SAFETY AND EFFICACY IN TREATMENT-NAIVE PATIENTS

Zimran, A., Durán, G., Mehta, A., Giraldo, P., Rosenbaum, H., Giona, F., Amato, D.J., Petakov, M., Terreros Muñoz, E., Solorio-Meza, S.E., Cooper, P.A., Chertkoff, R. and Brill-Almon, E. (Israel/Chile/UK/Spain/Italy/Canada/Serbia/Mexico/South Africa)

Professor Zimran updated us on this Taliglucerase clinical trial. Taliglucerase alfa is ERT approved for the treatment of adult patients with Gaucher disease. It is the first approved plant cell-expressed recombinant therapeutic protein.

The safety and efficacy of Taliglucerase alfa was studied in adult patients with Gaucher disease that had not been on treatment with ERT previously. Patients received Taliglucerase alfa 30U/kg or 60U/kg every other week for a total of 9 months in the first study, namely PB-06-001. The ones who completed this study were enrolled in the extension study, namely PB-06-003 and continued double-blind treatment for 36 months.

From the 32 patients in the PB-06-001 study, 29 completed it as 2 had hypersensitivity reactions and one became pregnant. In the PB-06-003 extension study, there were 26 patients enrolled. 3 stopped and 23 completed 36 months of treatment. Mean age was 35-38 years. Patients were severely affected at baseline with low haemoglobin and platelet concentrations, high chitotriosidase and spleen volumes 16 times the expected normal. Both Taliglucerase alfa doses caused at least a 50% reduction in spleen volume with the higher dose doing slightly better. All patients had normal haemoglobin concentrations at 24 and 36 months of treatment. Platelets were increased by 72% with the 60U/kg dose and by 40%with the 30U/kg dose.

In terms of safety all infusion-related reactions were mild to moderate. 3 severe adverse effects reported were not related to treatment.

In summary, the results of the longest multi-dose study of Taliglucerase show continuous improvement after 36 months of treatment with only mild and transient adverse events. Therefore, the safety and efficacy of Taliglucerase alfa is further supported.

ENGAGE: A PHASE 3, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, MULTI-CENTER STUDY TO INVESTIGATE THE EFFICACY AND SAFETY OF ELIGLUSTAT IN ADULTS WITH GAUCHER DISEASE TYPE 1: 18-MONTH RESULTS

Baris, H., Mistry, P.K., Lukina, E., Ben Turkia, H., Ghosn, M., Mehta, A., Petakov, M., Danda, S., Hadjiev, E., Angell, J., Ross, L. and Peterschmitt, M.J. (Israel/USA/Russia/Tunisia/Lebanon/UK/Serbia/India/Bulgaria)

Eliglustat is a novel oral treatment for Gaucher disease type 1. Eliglustat is a ceramide analogue and acts as an inhibitor of the enzyme glucosylceramide synthase that catalyzes the formation of glucocerebroside-like glycolipids.

Forty patients were randomized to placebo (N=20) or eliglustat (N=20) for 9 months. All patients were untreated; those already taking ERT were excluded. After 9 months 39 patients who completed the study, took eliglustat, in an open label

extension study for a further 9 month period. Mean age of the two groups was similar; most were of Caucasian origin and exhibited a diversity of mutations. In the first 9 month period, decrease in spleen size was observed with a moderate reduction in liver size and a significant increase in hemoglobin level and platelet count, all of which were statistically significant.

During the extension phase, sustained decrease in the spleen volume was shown in patients that continued on eliglustat. The decrease in liver volume was not as remarkable as the baseline liver was already smaller. Haemoglobin concentration increased; however, it was not too low at baseline. There was a remarkable increase in the platelet count in both arms.

Eliglustat was shown to be generally safe; as most adverse events were not severe.

In summary, the study met its primary and secondary efficacy endpoints. Results were clinically meaningful and statistically significant.

ENCORE—A RANDOMIZED, CONTROLLED, OPEN-LABEL, NON-INFERIORITY STUDY COMPARING ELIGLUSTAT TO IMIGLUCERASE IN GAUCHER DISEASE TYPE 1 PATIENTS STABILIZED ON ENZYME REPLACEMENT THERAPY: 24-MONTH RESULTS

Peterschmitt, M.J., Cox, T.M., Drelichman, G., Cravo, R., Balwani, M., Burrow, T.A., Martins, A.M., Lukina, E., Rosenbloom, B., Ross, L., Angell, J. and Puga, A.C. (USA/UK/Argentina/Brazil/ Russia)

Eliglustat is a novel oral treatment for Gaucher disease type 1. Eliglustat is a ceramide analogue and acts as an inhibitor of the enzyme glucosylceramide synthase that catalyzes the formation of glucocerebroside-like glycolipids.

This open-label, phase-3 trial (ENCORE, NCT00943111, sponsored by Genzyme, a Sanofi company) was designed to compare eliglustat with imiglucerase after 12 months of treatment in patients who had reached therapeutic goals with enzyme replacement therapy (ERT). Patients were randomized 2:1 eliglustat:imiglucerase (N=106 and N=53, respectively). The study aimed to demonstrate clinical stability in patients treated with eliglustat by measuring the percent of patients achieving the composite primary efficacy endpoint (stability of haemoglobin concentration, platelet count, and spleen and liver volumes). Results from the 12-month primary analysis period (PAP) and after the first 12 months of the Extension study are presented.

The patients were mostly of white Caucasian origin, with more females than males, and between 18-69 years of age. They had similar disease characteristics and were on ERT for a mean of 10 years previously before the start of the study.

At the end of the PAP, 94% of patients on imiglucerase and 85% of patients on eliglustat achieved the composite primary endpoint after 12 months. Therefore, non-inferiority of eliglustat to imiglucerase was demonstrated with respect to the composite endpoint. After 24 months, 87% of the eliglustat group (treated with eliglustat for 24 months) and 86% of the patients switched to eliglustat from imiglucerase (treated with eliglustat for 12 months) showed continued stability in spleen volume, liver volume, platelet count, and haemoglobin level. 145 of 159 patients originally treated in the study completed 24 months.

Mild adverse effects were reported with both treatments. More serious adverse effects included peripheral neuropathy and bowel obstruction.

In summary, the ENCORE study demonstrates that eliglustat is non-inferior to imiglucerase. It is well-tolerated, and after 24 months, 91% of the patients remain on the study.

COST-EFFECTIVENESS STUDIES IN GAUCHER AND FABRY DISEASE

Hollak, C., Biegstraaten, M., van Dussen, L., Rombach, S. and Dijkgraaf, M. (Netherlands)

Dr Hollak gave us a very interesting talk on cost-effectiveness studies applied in rare diseases. These studies were requested by the Netherlands for consideration of reimbursement of ERT for Fabry disease. This treatment has an annual cost of 200, 000 euros per patient per year, similar to ERT for Gaucher disease.

Under the orphan drug legislation, which came into effect in 2000 in the EU, most enzymes for treatment of lysosomal storage disorders were authorized 'under exceptional circumstances'. This means that the "real world" effectiveness of treatments is not always clear and may require structured follow-up. Drug-registries have limitations in this aspect, and data are usually not suitable for cost effectiveness studies.

Cost-effectiveness studies have many limitations as they are small in individual member states, there are no acceptable price limits and no consensus models shave been sufficiently developed for rare diseases. However, national governments request these studies in several member states to be able to justify reimbursement.

The cost-effectiveness studies on enzyme replacement therapies in Gaucher and Fabry disease conducted in the Netherlands serve as examples of what can be done at this time, but also show the limitations and need for further discussions.

For both disorders, a Markov model is developed, which includes separate disease stages, with utility scores (indicating quality of life) for each disease state. The advantage of treatment is assessed versus no treatment expressed as gain in quality adjusted life years (QALY). For ERT with similar annual costs, the cost per QALY is highly dependent on the effectiveness of treatment. Within this model, ERT for Gaucher disease is estimated to be roughly 7 times more effective than ERT for Fabry disease. ERT for Gaucher disease increases the years free of end organ damage by almost 13 years. There are many limitations to these studies, mainly related to the small study populations and heterogenicity of the patients.

There is an increasing need to develop disease, rather than drug registries for assessment of " real life" effectiveness, including cost-effectiveness analysis. Responsibility for treatment should be shared between academia, patients, regulators and the industry to make these rare and expensive treatments sustainable so that patients have equal access to effective treatment.

NOVEL TREATMENT FOR GAUCHER DISEASE – ORAL ADMINISTRATION OF PLANT CELLS EXPRESSING GCD: PHASE 1 STUDY RESULTS AND PHASE 2A PROGRAM

Zimran, A., Rosenbaum, H., Golembo, M., Velitski, S., Kivity, V., Chertkoff, R., Almon, E. and Shaaltiel, Y. (Israel)

Professor Zimran gave us an exciting glimpse of this novel and revolutionary oral therapy for Gaucher disease by describing the preliminary result of ongoing clinical trials. It is revolutionary as it involves the oral delivery of therapeutic proteins. This has clearly been the ultimate goal since the invention of intravenous ERT for Gaucher disease. Fundamental problems with the stability of the protein, its enzymatic degradation and ineffective absorption have made this an unrealistic goal as there has been limited success to date in the development of oral therapy.

However, hope now emerges in Israel, where this oral therapy is currently in clinical trials. Taliglucerase alfa, which is an FDA approved ERT for Gaucher disease

is expressed in carrot cells which may provide a natural capsule to protect the ERT form degradation by the stomach due to the rigid plant cell wall. The edible carrot cells expressing GCD provide enzyme that is 'ready to use' as it requires no additional modification to maximise cellular uptake. These are the same cells that produce Taliglucerase alfa. Once in the blood, the enzyme is expected to act in the same way as the intravenous form of Taliglucerase alfa. The oral formula is named PRX-112.

Increasing concentration of active prGCD has been shown in the liver and spleen after oral administration. The PRX-112 Phase 1 study looks at the safety and pharmacokinetics of the drug. Adult Gaucher disease patients were recruited and 3 dose groups selected for oral administration of the PRX-112 cell suspensions. The treatment was well tolerated. There were no adverse events and no detectable antibodies. No patient discontinued the trial.

Monocytes represent a surrogate of GD target cells. Following oral administration of PRX-112:

Active enzyme is detected in the patient's blood (proof of concept)

There is increase in Cmax and active GCD (evidence for absorption)

The pharmacokinetic profile is different to that of intravenous ERT but shows continuous secretion for over 30hours as opposed to minutes

A meaningful increase in platelet count was also shown in some patients.

A Phase 2A, 28 day, dose escalation safety and pharmacokinetics study in 10 treatment naive adult patients is now underway. 5 daily doses of PRX-112 are being used over a month and the pharmacokinetics or the drug in the plasma and leucocytes assessed on day 4 of each dosing week for 24 hours. Anti-prGCD antibodies are determined at baseline, D15 and 1 week after the end of the treatment period.

A Phase 2B, 6 month clinical trial is planned to demonstrate whether defined endpoints are achieved. This will be followed by a Phase 3 switch-over 12 month study with stable patients on ERT switching to PRX-112.

IDENTIFICATION OF MICRORNAS THAT MODULATE GLUCOCEREBROSIDASE ACTIVITY IN GAUCHER DISEASE CELLS

Siebert, M., Westbroek, W., Chen, Y-C., Moaven, N., Li, Y., Velayati, A., Saraiva-Pereira, M.L., Martin, S. and Sidransky, E. (USA/Brazil)

Dr Siebert presented a very interesting paper on the role of microRNAs in Gaucher disease with a view to them being considered a potential novel therapeutic approach to increase the levels of glucocerebrosidase (GCase).

microRNAs (miRNAs) are small RNA molecules that do not encode a protein sequence and are involved in many diseases. The role of miRNAs in Gaucher disease (GD) was explored as the manifestations of Gaucher disease in an individual (the phenotype) cannot always be predicted by the genetic mutation that the individual patient carries.

To look at miRNA effects on GCase activity, a screen of 875 miRNAs was performed in GD fibroblasts and GCase activity measured. Some up-regulated and some down-regulated GCase activity. miR-195-5p and miR-16-5p both increased GCase expression and levels. These miRNAs act through RNA activation, mRNA stabilization or regulation of transcription factors. miR-127-5p did not affect GCase expression, but it down-regulated GCase activity through LIMP-2. LIMP-2 is a receptor on the lysosome for the proper trafficking of GCase from its site of production (the endoplasmic reticulum) to its site of action (the lysosome). Cells that were treated with this miRNA secreted GCase into the extracellular medium.

miRNAs alter GCase activity in GD patient cells and may be responsible for modifying disease manifestations in patients with the same mutations. They are another member of a group of molecules called modifiers and can be a potential future therapeutic target.

GAUCHER DISEASE AND PARKINSONISM: LONGITUDINAL CLINICAL CHARACTERIZATION AND PROGNOSIS

Grisel Lopez [1], Jenny Kim [1], Catherine Groden [2], Edythe Wiggs [3], Nahid Tayebi [1], Ashley Gonzalez [1], Ellen Sidransky [1]

[1]National Human Genome Research Institute (NHGRI), Medical Genetics Branch, Section of Neurogenetics, National Institutes of Health (NIH), Bethesda MD, USA

[2]NHGRI, Undiagnosed Disease Program, NIH, Bethesda MD, USA

[3]National Institutes of Neurological Disorders and Stroke, NIH, Bethesda MD, USA

Corresponding author: Ellen Sidransky, M.D.

Objective: Longitudinal evaluation of patients with Gaucher disease and Parkinson disease in the Genetics Clinic at NHGRI for phenotypic characterization and prognosis.

Background: Mutations in the glucocerebrosidase (*GBA*) gene are the most common genetic risk factor associated with parkinsonism. The clinical presentation can be indistinguishable from classic Parkinson disease (PD) or patients can have features of atypical parkinsonian syndromes such as Dementia with Lewy bodies.

Methods: Individuals with both GD and PD were followed at NHGRI Genetic Clinic. Parameters including age of onset of GD symptoms, initial symptoms, use of enzyme replacement or substrate reduction therapy, genotype, severity, age of onset of PD symptoms, use of anti-parkinsonian medications, severity, disease duration, UPSIT score, age at death, and neurocognitive profile were analyzed.

Results: Patients with GD and PD have an earlier onset of PD symptoms. PD disease duration did not differ significantly from sporadic PD patients. Neurocognitive dysfunction was frequent, consistent with findings in the literature. There was no correlation between PD severity and *GBA* genotype, type or duration of Gaucher therapy and GD symptoms.

Conclusions: Disease duration in patients with GD and PD is similar to that seen in patients with sporadic PD. Extent and severity of PD symptoms varied significantly the mean age at death was younger, as expected with an earlier disease onset. Cognitive dysfunction including lack of concentration, short-term memory problems, and slow processing speed were frequently confirmed. Continued longitudinal follow-up of cohorts with GD and PD is essential for establishing the efficacy of any therapeutic interventions.

COMPARISON OF PARKINSON RISK IN ASHKENAZI JEWISH PATIENTS WITH GAUCHER DISEASE AND AND *GBA* HETEROZYGOTES

Dinur, T., Quinn, T., Sakanaka, K., Levy, O., Waters, C., Fahn, S., Dorovski, T., Chung, W.K., Pauciulo, M., Nichols, W., Rana, H.Q., Balwani, M., Bier, L., Elstein, D., Zimran, A. and Alcalay, R.N. (Israel/USA)

The aim of this study was to estimate the age-specific risk of PD in Ashkenazi Jewish (AJ) patients with Type-1 GD and in *GBA* obligate carriers.

The Information on age-specific risk for Parkinson (PD) in both Gaucher disease (GD) patients and glucocerebrosidase *(GBA)* carriers is important for counseling these populations.

Some data on the *GBA* Epidemiology :

Frequency of *GBA* mutations in Ashkenazi Jews (AJ) is estimated at 1:17 (~6%), Frequency of GD among AJ is estimated at 1:850 (~0.001%), Prevalence of PD in AJ is around 1-2% Among PD patients, at least 15% of AJ and 3% of non-AJ carry one of the two most common *GBA* mutations (N370S and L444P). 4.2% of PD autopsy subjects in England carried *GBA* mutations. Roughly 1/3 of all AJ with PD in the USA and Israel are carriers of either *GBA* or LRRK2 mutations.

The Risk for PD in Gaucher Disease Patients, According to the ICGG Registry: The probability that a patient with Type 1 GD will develop Parkinsonism (Rosenbloom 2011): by age 70: 5-7% ,by age 80: 9-12%.

Other Studies: 11/444 (2.5%) USA Type 1 GD dx. with PD (Bultron, 2010), 11/510 (2.2%) Israel Type 1 GD dx. with PD (Ben Chetrit, 2012), and 1/75 (1.3%) Dutch Type 1 GD dx. with PD (Biegstraaten, 2008)).

Our design study: Participants were recruited from two major Gaucher centers: Shaare Zedek Medical Center , Jerusalem, Israel – 332 GD patients, and Mount Sinai School of Medicine, NY, USA - 95 GD patients. AJ Non *GBA* carriers / non PD (n = 77) controls were spouses of PD patients at Columbia University Medical Center, NY. All were adult AJ (age ≥ 18 years) and had AJ grandparents. Most GD patients (97.9%) carried at least one N370S mutation.

The table below shows: **The age-specific risk (penetrance) for PD among AJ with and without *GBA* mutations:**

	Age 60	Age 70	Age 80
Non-carriers (n=154)	0.7% ±1.37%	0.7% ±1.37%	2.1% ±2.94%
Obligate N370S carriers (n=464)	1.2% ±0.98%	3.5% ±1.96%	5.9% ±3.14%
Obligate *GBA* carriers (n=694)	1.5% ±0.98%	5.6% ±1.96%	7.7% ±2.74%
Gaucher patients (N370S homozygote or compound heterozygote carriers), (n=427)	4.7% ±3.33%	9.1% ±6.07%	9.1% ±6.07%

Our age-specific estimation of PD risk among GD patients (9.1% by age 80) is similar to estimations derived from the International International Gaucher Group (ICGG) Registry data (9-12% by age 80) (Rosenbloom 2011).

Our estimations of *GBA* carriers' risk (n=694) for PD (7.7% by age 80) is lower than the reports from Europe: UK (n=166) - 15% by age 80 (McNeill et al, 2012),and France (n=525) - 29.7% by age 80 (Anheim et al, 2012).

What are the reasons for the large difference between these Studies and ours? Differences in methodology-*GBA* carriers ascertainment:
We collected data on carriers regardless of family history of PD, whereas the UK and France studies were all ascertained through a familial PD cohort. Difference in *GBA* mutations: milder mutations among AJ (N370S -our study) and more severe (non N370S) in UK & France.

Comparison of *GBA* carriers with PD versus Gaucher patients with PD: Mean age onset Parkinson is later in obligate carriers than in GD patients (65 versus 54 years, p=0.003) .No male predominance in obligate carriers (53% versus 73%).

However, by age 80, the difference in the age-specific risk of PD between the two groups had diminished (9.1% in GD patients and 7.7% in carriers).

While both *GBA* carriers and GD patients are at an increased risk for PD, the rather similar magnitude of PD risk by age 80 suggests that penetrance of PD in these populations is not exclusively a result of *GBA* enzymatic deficiency.

Despite the fact that *GBA* mutations are among the most common genetic risk factors for PD, our results (as well as others) indicate that most GD patients and *GBA* carriers will never develop PD.

In order to refine the estimation of the age-specific risk of PD in these populations and to identify genetic and environmental risk modifiers, long term follow up of GD patients and *GBA* carriers is required.

DISRUPTION OF MONOAMINE METABOLISM IN LYSOSOMAL STORAGE DISORDERS: A MECHANISTIC LINK TO PARKINSON'S DISEASE?

Heales, S., Burke, D., Neergheen, V. and Pope, S. (UK)

Loss of the neurotransmitter dopamine is well documented to occur in Parkinson's disease (PD). It is now becoming clear that lysosomal disorders (LSDs) such as Gaucher disease (GD) are associated with an increased risk of developing PD.

By measuring neurotransmitters in the CSF, Prof Simon Heales at Great Ormond Street Hospital / UCL Institute of Child Health in London, has showed impaired dopamine metabolism in GD and other LSDs. Currently mechanisms responsible for the loss of dopamine function are not known.

Using models of GD, Derek Burke (a PhD student of Simon's) has been looking at two enzymes; GBA1 and GBA2. GBA1 is known to be deficient in GD. Recent findings, by Derek, have shown that GBA2 activity is particularly high in the brain and that its activity can become even higher when GBA1 activity is compromised. Turning to patients, data is now emerging to indicate that not all individuals have the ability to increase GBA2 activity in response to loss of GBA1. The group is now working to ascertain whether GBA2 influences the progression of GD and the risk of developing PD.

THE CONNECTION BETWEEN ERAD, UPR, GAUCHER DISEASE AND PARKINSON'S DISEASE

Horowitz, M., Braunstein, H., Enoch, M. and Maor, G. (Israel)

GD patients and carriers of GD mutations have a significantly higher risk to develop Parkinson disease (PD) in comparison to the general population. This implies that the presence of a mutant GBA allele is a dominant predisposing factor that causes PD in one of two ways: 1) Haploinsufficiency which means that one allele can cause disease by not producing enough enzyme in neuronal cells and subsequent substrate accumulation. This is not the case, as the carriers do not accumulate substrate. 2) Gain of function, which means that the new gene product, i.e the mutated glucocerebrosidase (GCase) has a deleterious gain of function.

Professor Horowitz described her very exciting, state-of-art research on the pathogenesis of PD in GD, based on the second hypothesis. The hypothetical model is that misfolded mutant (faulty) GCase leads to retention inside the Endoplasmic Reticulum (ER) and triggering of the ER stress response called the Unfolded Protein Response (UPR). Mutant GCase moves from the ER to the cytoplasm and undergoes there ubiquitination and degradation. Parkin mediates ubiquitination of mutant GCase. While doing so, there is accumulation of other parkin substrates some of which are pathogenic to cells, like PARIS.

Animal models in use to study the carrier state of Gaucher disease were not suitable to study the association between UPR, GD and PD. *Drosophila* was identified as a possible animal model as it has two GBA1 genes. The GBA genes on chromosome 3 of the *Drosophila* encode GCases that have a 50% similarity to the human protein with conserved glycosylation sites and a fully conserved active site. There are two fly lines defective in the two GBA genes and in the Horowitz lab other fly lines were produced which express human mutant GBA alleles. All mutant flies, carrying either the *Drosophila* or the human GBA mutant alleles exhibit Parkinsonian phenotype. This includes death of dopaminergic neurons in the brain and reduced ability to climb. When the flies are treated with ambroxol, which removes the mutant enzyme from the ER, there is reduced loss of dopaminergic cells and improved fly climbing activity.

VISUALISATION OF ACTIVE GLUCOCEREBROSIDASES IN RODENT BRAIN WITH HIGH SPATIAL RESOLUTION BY FLUORESCENT ACTIVITY BASED PROBES

Herrera Moro Chao, D., Kallemeijn, W.W., Orre, M., Ottenhoff, R., Van Roomen, C., Foppen, E., Renner, M., Marques, A., Moeton, M., Siljee, J., Van Eijk, M., Boot, R., Kalsbeek, A. and Aerts, J.M.F.G. (Netherlands)

The link between Gaucher disease and neurodegenerative diseases, such as Parkinsonism, is now well established. As a result, lysosomal glucocerebrosidase (GBA) and cytosolic glucosylceramidase 2 (GBA2) have attracted a lot of interest and their distribution in the brain is a subject of intense research with a view to decipher their role in the pathogenesis of the alpha-synucleinopathies.

Inhibodies are molecules that allow labelling of active GBA1 only. Other molecules, called anybodies bind to many similar enzymes including GBA1, GBA2, GBA3. They bind to the enzymes as irreversible inhibitors. As systemic administration does not cross the blood-brain barrier and therefore does not penetrate the brain, these need to be given via the intracerebro-ventricular route to label the active enzyme in the brain.

The researchers gave a constant infusion of the probes through the lateral ventricles of the brain. The results showed GBA1 co-localising with lysosomal proteins and higher GBA activity in the basal ganglia and brainstem (motor areas of the brain) as opposed to the hippocampus and the motivational cortex.

Using the right probe to examine different cell types, the group showed that astrocytes from the hindbrain present higher GBA1 activity that microglia. Active GBA2 was shown to be higher in the cerebellar cortex.

In summary, the location of active GBA1 predominantly in the motor areas and of active GBA2 in a cerebellar distribution would be in keeping with a role in disorders like Parkinson and Ataxias.

THE EFFECT OF PROTEASE INHIBITION ON LYSOSOMAL GLUCOCEREBROSIDASE

Boot, R.G., Oussoren, S.V., Scheij, S., Ferraz, M.J. and Aerts, J.M.F.G (Netherlands)

The breakdown of complex glycolipids and glucosylceramide (GlcCer) occurs in a stepwise manner in the endosomes and lysosomes. As we know GlcCer is broken down into ceramide and glucose by beta glucocerebrosidase (GBA). There are 3 types: GBA1 located inside lysosomes, GBA2 located in the plasma/ER membrane and a cytosolic enzyme, GBA3. In Gaucher disease, we know there is little GBA1 activity, therefore GlcCer builds up in lysosomes, especially in macrophages.

Cathepsins, are proteases that are involved in protein turnover that have been shown to be high in tissues from GD patients. If one of them is involved in GBA1 breakdown and we manage to stop it from doing its job, then there will be more GBA around in the lysosome.

The researchers used leupeptin and fluorescent activity based probes to label GBA and visualise its lifecycle. They showed that active GBA in vitro increases in the presence of leupeptin in both healthy and GD cells.

Using special activity-based probes for cysteine proteases, that are enzyme inhibitors that label active Cathepsins, the researchers have shown that inhibition of either cathepsins B, S or both results in the most stable GBA in a dose-dependent fashion.

This is an area of ongoing active research to try and evaluate the role of cathepsins as a therapeutic target in GD

APPROACH TO DIAGNOSIS AND TREATMENT OF OSTEOPOROSIS – STATE OF THE ART

Ish-Shalom, S. (Israel)

Dr Sofia Ish Shalom gave us a very detailed talk on the latest osteoporosis diagnosis and management using published data from the latest clinical trials in the field.

Osteoporosis is a skeletal disorder characterised by compromised bone strength with an increased risk of fractures at characteristic skeletal sites: T4 to L5 vertebra, distal radius, proximal humerus and proximal femur. The fractures may happen after a minimal trauma, such as falling from a standing height or without any trauma (vertebral compression fractures). The disease affects one in 3 women after menopause and one in seven men after the age of 50.

Initially the diagnosis was based solely on low bone mineral density (BMD), usually assessed with a DXA scan, a dual-energy x-ray absorptiometry scan. It works on the principle that the denser the bone the less photon flow will go through current criteria for densitometric diagnosis of osteoporosis in postmenopausal women and in men above the age of 50, are based on standard deviation scores (T score) from the mean of healthy, gender matched 30 years old: 0 to -1 is defined as normal BMD; -1 to -2.5 –osteopenia; ≤-2.5-osteoporosis.

Since most of the osteoporotic fractures happen in individuals with osteopenic BMD, low BMD constitutes a quantifiable risk factor for a disease and it is not a disease itself. Recently several fracture risk calculators were developed. The WHO approved FRAX that is country-specific and includes clinical factors such as age, BMI, previous fracture, parental hip fracture, glucocorticoid use, smoking, alcohol use and several concomitant diseases. Fracture risk can be calculated based on clinical factors, with or without BMD measurement.

Treatment of osteoporosis is aimed at fracture risk reduction. It consists of fall prevention strategies, improvement of muscle function by adequate physical activity, adequate nutrition with calcium and vitamin D supplementation, when necessary, and medications.

Two categories of medications are used, antiresorbing agents (oral and intravenous bisphosphonates, raloxifen, denosumab) and one anabolic agent (recombinant PTH 1-34 –teriparatide).The antiresorbing agents decrease bone resorption with a concomitant decrease in bone formation, since there is a physiologic coupling between the two processes. This leads to a decrease in fracture risk between 25 to 60% at various skeletal sites. Anti-resorbing agents are usually used as first line treatment in newly diagnosed patients with osteoporosis. Teriparatide is usually used in patients with severe osteoporosis, meaning patients with osteoporotic fractures, especially in patients that sustained an osteoporotic fracture while treated with an antiresorbing agent. Side effects include upper GI problems with oral bisphosphonates, and a rare but disconcerting problem of osteonecrosis of the jaw and atypical femoral fractures with all potent antiresorbing agents.

Assessment of therapeutic response in an individual patient is problematic since BMD explains only 4-24% of the treatment effect of most antiresorbing agents and the observed increase in BMD during treatment is close and often below the least significant difference between consecutive measurements.

Bone turnover markers have been used for many years in the assessment of skeletal response to therapeutic agents in osteoporosis. Recently two of them have been approved by the International Osteoporosis Foundation (IOF) due to their analytical stability. These are collagen type I synthesis and degradation products. Propeptide of type I procollagen(PINP), is produced during collagen formation and can be measured in serum. It has been selected as the reference marker for bone formation due to its robust nature and dynamic response to anabolic treatment . As it is metabolised in the liver, its clearance is not affected by renal function.

Collagen Type I C-Telopeptide (CTX) is a bone resorption marker that has a circadian rhythm (more pronounced for markers of bone resorption) being higher in the early morning and dropping during the late afternoon.

In spite of the fact that diagnostic and therapeutic tools for osteoporosis are readily available, 8 out of 10 patients do not receive treatment during the first year after an osteoporotic fracture.

Chronic glucocorticoid s (GC) use is an important cause of osteoporosis, 50 % of chronic GC users are affected by

osteoporosis. GC initially increase bone resorption, followed by decreased osteaoblast and osteocyte survival, leading to an uncoupling between bone resorption and bone formation with a subsequent micostructural deterioration of bone and increased risk of fractures. In a head to head study that compared the effect of teriparatide (an anabolic agent) with alendronate (an antiresorbing agent), on fracture prevention in GC treated patient, anabolic treatment was proved to be significantly more efficient

In GD there are many unresolved issues that are related to bone disease. We know there is a high risk for fractures at diagnosis and increased risk of aseptic necrosis of bone. Comorbidities are clearly important. There is a need for development of reliable tools to assess therapeutic response and studies to guide us as to the optimal and safe duration of treatment with antiresorbing agents.

BONE ASSESTMENT IN SPANISH TYPE 1 GAUCHER DISEASE PATIENTS: THE ZAGAL PROJECT

Andrade, M., Alfonso, P., Irun, P.,Garcia-Frade, J., Lorenzo, J.F., Luño, E., Martin,.E., Puerta, J., de la Serna, J., Villalon, L., Pocovi, M., Roca, M. and Giraldo, P. (Spain)

The study aimed to assess bone disease in GD patients after two and five years of treatment with miglustat, using an ultrasound technique. Osteopenia and densitometry have been assessed.

The ZAGAL project comprised 63 patients, both male and female, with moderate and severe bone pain. 35 patients were evaluated with both MRI and ultrasound.

After two years of therapy, improvement in chronic bone pain has been reported. A constant progress was registered after five years and bone marrow burden increased.

This study indicated that ultrasound combined with MRI is a very useful tool to evaluate bone assessment and monitor BMB and BMD.

CHANGES IN PERIPHERAL BLOOD OSTEOCLAST CULTURES IN RELATION TO FEATURES OF BONE DISEASE IN ADULT GD1 PATIENTS

Hughes, D.A., Cunningham, N., Bauernfreund, Y., Reed, M. and Mehta, A.B. (UK)

There is still an incomplete understanding of mechanisms of bone disease in GD affected patients. Skeletal pathologies in GD have diverse manifestations (bone pain, avascular necrosis, reduced bone density).

Previous studies have been done in order to identify markers of bone degradation and formation. Osteoclasts derived from peripheral blood of GD patients were more active than control cells: Osteoclast production was enhanced and bone degradation increased.

This study aimed to set up an *in vitro* assay that can be used to follow the changes in clinical features in GD patients suffering from bone disease. In particular, the relationship between bone disease and the number of osteoclasts was examined.

68 patients have been followed for 10 years and all clinical symptoms were collected. Patients who received treatment and whose biological features improved revealed a reduction in osteoclasts in response to the therapy.

To conclude, the research showed that *in vitro* generation of osteoclasts was an important tool for monitoring ameliorations in bone disease in response to ERT in GD patients.

LIMITS ON USE OF HEALTH ECONOMIC ASSESSMENTS FOR RARE DISEASES

Roos, J.C.P., Hyry, H.I., Stern, A.D. and Cox, T.M. (UK)

National parliaments have created incentives for developing orphan drugs, but treatments are very expensive as there is no price cap in the regulations which give a monopoly to the drug producer. Governments try to ensure money is spent wisely and often base decisions about whether to fund a treatment on efficiency measures comparing cost and the quality and length of life gained.

This study analysed the reliability of such cost-effectiveness methods from a legal and ethical point of view and identified some potentially fixable problems with this approach. These include aspects related to the arbitrary cost threshold set, the blindness to prejudice and time consistency bias, the limitation of inputs and opacity in the calculus. However we also identified some issues with cost efficiency which makes them unsuitable for use in rare diseases: these include the difficulty in valuing another's quality of life, degrading human life by pricing it, excluding market dynamics, addressing humans as location of utility and evaluating costs with moral arbitrariness.

We show that health economic models have many limits and are inadequate as the only basis for making decisions about health. However, they can be useful when several competing treatments are analysed for a condition. Please see our article in the Quarterly Journal of Medicine 2014 for the detailed explanation of the limits of the cost-effectiveness approach in rare diseases.

THE LEGAL IMPERATIVE FOR TREATING RARE DISORDERS

Hyry,H.I., Roos, J.C.P., Manuel, J. and Cox, T.M. (UK)

Orphan drugs can be very costly but not treating people affected by rare disease carries both human and economic tolls. This study used the United Kingdom as an example to review the legal arguments for why governments and other healthcare providers must provide these treatments despite their high cost. These include disability legislation, national and organisational constitutions, judicial review, tort law (a gap-filling area of the law which addresses situations not captured by contract law or criminal law) and human rights legislation. These taken together provide a compelling set of arguments for why these drugs must be funded. Those with disabilities may not be discriminated against and public bodies must therefore consider how policies affect people with disabilities and eliminate unlawful discrimination and advance equality. Our legal framework will assist doctors and patients in ensuring the continued treatment despite significant economic pressure to reduce funding. The legal right to treatment extends beyond rare diseases and our analysis may therefore affect allocation of healthcare budgets throughout the EU.

New European Working Group on Gaucher Disease (EWGGD) Board

At the 11th EWGGD in Haifa a new Board was elected for the period 2014-2016:

Prof. Dr. med. Stephan vom Dahl : ***chairman***
Dept. of Gastroenterology, Hepatology and Infectious Diseases
University Hospital Duesseldorf
Moorenstrasse 5
40225 Duesseldorf
Germany
Tel: +49 211 81 19648
Fax: +49 211 81 17517
Email: stephan.vomdahl@med.uni-duesseldorf.de

Dr. Helen Michelakakis : ***vice-chairman***
Department of Enzymology and Cellular Function
Institute of Child Health
"Ag. Sophia" Children's Hospital
11527 Athens
Greece
Tel: +30 213 2037320
Fax: +30 210 7700111
Email: ecfdept@ich.gr or hel.mikaki@gmail.com

Prof. Dr. Hans Aerts : ***founder president***
Department of Medical Biochemistry, K1-110
Academic Medical Center,
University of Amsterdam
PO box 22700
1100 DE Amsterdam
The Netherlands
Tel: +31 20 5665156
Email: j.m.aerts@amc.uva.nl

Dr. Mia Horowitz : ***board member***
Department of Cell Research and Immunology
Tel Aviv University
Ramat Aviv, 69978
Israel
Tel: +972 3 6409285
Fax: +972 3 6422046
Email: horwitzm@post.tau.ac.il

Dr. Nadia Belmatoug : ***board member***
Centre de Référence des Maladies Lysosomales
Hôpitaux Universitaires Paris Nord Val de Seine, site Beaujon
100 Bd du Général Leclerc 92110 Clichy
France
Tel: +33140875286
Fax: +33140874434
Email: nadia.belmatoug@bjn.aphp.fr

Dr. Hanna Rosenbaum : ***board member***
Hematology and Bone Marrow Transplantation Department
Rambam Health Care Campus
Haifa
Israel
Tel: +972 4 8542 541
Fax: +972 4 8541 514
Email: h_rosenbaum@rambam.health.gov.il

Prof. Dr. Elena Lukina :
host president of the 2016 meeting
Department of Orphan Diseases
Hematology Research Center
4, Noviy Zykovsky pr.
125167, Moscow
Russia
Tel/fax: +7 495 612 0923
E-mail: elenalukina02@gmail.com

Jeremy Manuel OBE : ***board member***
Chairman European Gaucher Alliance (EGA)
Evesham House Business Centre
48-52 Silver Street
Dursley
Gloucestershire
GL11 4ND
UK
Tel: +44 1453 549231
Fax: +44 1453 549231
Jeremy@eurogaucher.org
www.eurogaucher.org

Dr. Marieke Biegstraaten : ***secretary***
Department of Internal Medicine,
Division of Endocrinology and Metabolism, F5-166
Academic Medical Center,
University of Amsterdam
PO box 22700
1100 DE Amsterdam
The Netherlands
Tel: +31 20 5668315
Fax: +31 20 6917682
Email: m.biegstraaten@amc.uva.nl

EWGGD
European Working Group on Gaucher Disease

Preliminary Announcement

The 12th European Working Group on Gaucher Disease (EWGGD) meeting will be held in Moscow, Russia in June 2016 (dates to be confirmed) hosted by Elena Lukina

For further information please contact:
Dr Marieke Biegstraaten (secretary)
m.biegstraaten@amc.uva.nl

About the European Gaucher Alliance (EGA)

The EGA is a pan-European umbrella group representing the interest of Gaucher patients and those of not-for-profit Gaucher patient groups throughout Europe and elsewhere in the world.

The aims and objectives of the EGA are:

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

Members of the Management Council

A new board of directors was elected at the EGA meeting on 24th June 2014 in Haifa, Israel.

The members elected were:

1. Vesna Aleksovska (Macedonia)
2. Anne-Grethe Lauridsen (Denmark)
3. Jeremy Manuel OBE (UK) - Chairman
4. Pascal Niemeyer (Germany)
5. Johanna Parkkinen (Finland)
6. Sandra Zariņa (Latvia)
7. Irena Žnidar (Slovenia)

Chief Executive Officer: Tanya Collin-Histed (UK)

Membership

Although the EGA is a European organisation, it has always accepted responsibility to help and support Gaucher patients and patient groups from all parts of the world and aims to continue in this role.

Beside the original founding associations of the EGA (the UK, Italy, Netherlands, Israel, France and Sweden), full membership is available to all European 'not for profit' Gaucher organisations or umbrella groups representing the interest of patients suffering from rare diseases, which are under the control of patients.

Patient groups from non-European countries can apply to be associate members of the EGA, but will not have voting rights.

EGA member countries:

Austria; Belgium; Bosnia & Herzegovina; Bulgaria; Canada; Croatia; Czech Republic; Denmark; Estonia; Finland; France; Germany; Greece; India; Ireland; Israel; Italy; Jordan; Latvia; Lithuania; Luxembourg; Macedonia; Mexico; Moldova; Netherlands; Norway; Paraguay; Poland; Romania; Russia; Serbia; Slovakia; Slovenia; South Africa; Spain; Sweden; Switzerland; Ukraine; UK; USA

For more information, or to join the EGA, please contact any of the Council Members known to you or email Tanya Collin-Histed (Chief Executive Officer) tanya@eurogaucher.org or Jo Cook (Projects Co-ordinator) jo@eurogaucher.org.