International Scientific Congress
on Spinal Muscular Atrophy

Kraków, 25–27 January 2018

SMA Europe

fundacja SMA

Programme & Abstract Book

It is with great pleasure that we welcome you to Kraków for our International Scientific Congress on spinal muscular atrophy, lead by SMA Europe, with the support of FSMA Poland.

Spinal muscular atrophy is experiencing exceptional times. Research has progressed significantly, clinical trials have multiplied, new treatments are coming on the market.

It was therefore important for us, as representatives of SMA patients, to bring together in Europe, all scientists in the field. Innovative therapies and new forms of care are essential to all of us, especially the sick and their families, who fight the disease on a daily basis.

More broadly, this development, these results, show that it really is possible to accelerate the development of treatments by bringing together researchers and clinicians. The best experts in this emerging discipline, from all five continents are here to compare their latest work and discoveries not only in basic, but also in clinical research and therapies.

We all have a very high level of expectation, and I wish you a very nice and informative congress.

Marie-Christine Ouillade
SMA Europe President

WELCOME

It is highly gratifying to welcome you to the first European congress dedicated solely to spinal muscular atrophyin the lovely Polish city of Kraków.
Since the genetics of SMA was first identified in 1995 science has made tremendous progress. Hundreds of researchers devoted their efforts to elucidate this deadly disease, its mechanisms and possible treatments, andacademic journals have published thousands of papers on SMA.
As our understanding of the disease grows, it becomes necessary to sum up ourknowledge, share it and, all together, look at the road laying ahead. With this in mind, in 2016 SMA Europe embarked on a task of convening an international scientific congress dedicated solely to spinal muscular atrophy. SMA Foundation Poland, the national SMA patient organisation in Poland, volunteered to host it, considering it also a unique opportunity for the research and medical community in this part of Europe.
To make such an event reality required tremendous effort of many people. Here I would like to express my sincere gratitude to everybody who has made the Congress possible: the Scientific Committee who spent days and nights working on the content; Rector of the Jagiellonian University Professor Wojciech Nowak and the Chair of the Clinic of Orthopaedics and Rehabilitation Professor Maciej Tęsiorowski for hosting the event at the University; the Sponsors who understood the needof the time and responded generously; the Working Group of SMA Europe and the Volunteers of SMA Foundation Poland – in particular Marie-Christine, Vanessa, Maria, Gosia and Kasia – who worked countless hours of day and night to make the event great; to Beata and Kasia from the Trip Group, our Logistic Partners, for top-notch organisation; and to all our Media Partners who made sure the news of event reaches far and wide.
I hope that all of you the Esteemed Participants will remember the Congress as a worthwhile event and return home with plenty of good memories of Kraków.

Kacper Rucinski
Co-Founder and President
SMA Foundation Poland

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Schedule of events

Thursday, 25th January 2018

Lunch	12.00 – 12.55	Level 2, Exhibition Room
Welcome	12.30 – 13.45	Level 0, Large Hall A
Keynote	13.45 – 14.30	Level 0, Large Hall A
Session 1	14.30 – 15.30	Level 0, Large Hall A
Coffee Break	15.30 – 16.00	Level 2, Exhibition Room
Session 2	16.00 – 17.30	Level 0, Large Hall A
Poster session A	17.30 – 19.00	Level 2, Holl, Exhibition Room
Reception	19.00 – 21.30	Level 2, Exhibition Room

Friday, 26th January 2018

Novartis symposium	08.00 – 09.00	Level 0, Medium Hall AB
Session 3	09.00 – 10.30	Level 0, Large Hall A
Coffee Break	10.30 – 11.00	Level 2, Exhibition Room
Session 4	11.00 – 12.30	Level 0, Large Hall A
Lunch	12.30 – 13.00	Level 2, Exhibition Room
Avexis symposium	13.00 – 14.00	Level 0, Medium Hall AB
Session 5	14.00 – 15.30	Level 0, Large Hall A
Coffee Break	15.30 – 16.00	Level 2, Exhibition Room
Poster Session B	15.30 – 16.30	Level 2, Exhibition Room
Workshop 1	16.30 – 17.30	Level 0, Medium Hall A
Workshop 2	16.30 – 17.30	Level 0, Medium Hall B
Magic trip (faculty)	17.30 – 19.00	Wieliczka Salt Mine
Gala dinner (faculty)	19.00 – 22.00	Wieliczka Salt Mine

Saturday, 27th January 2018

Biogen symposium	08.00 – 09.00	Level 0, Medium Hall AB
Session 6	09.00 – 10.30	Level 0, Large Hall A
Coffee Break	10.30 – 11.00	Level 2, Exhibition Room
Session 7	11.00 – 12.30	Level 0, Large Hall A
Conclusion	12.30 – 13.00	Level 0, Large Hall A
Awards & closing ceremony	13.00 – 13.30	Level 0, Large Hall A
Lunch	13.30 – 14.30	Level 2, Exhibition Room

SMA Europe

About SMA Europe

SMA Europe is a pan-European patient organisation which offers awareness-raising, knowledge and expertise of the rare neuro-muscular disease: spinal muscular atrophy (SMA), for the benefit of people living with SMA and their families/carers. SMA Europe also funds research into the condition and into potential therapies.

The organisation

SMA Europe was founded in 2006 by members of 6 organisations from France, Germany, Italy, Spain, the Netherlands and the UK, to organise and drive common actions for spinal muscular atrophy (SMA) at European level.

They recognised a number of opportunities for collaboration in the field of SMA. In particular: a shared commitment to advance translational research for SMA; an agreement on the need for a new level of interaction and collaboration among several of the European leading organisations targeted to the understanding and treatments/ or cure for SMA.

Since 2006, SMA Europe has grown its membership and has welcomed organisations from Iceland, Poland, Romania, Russia, Sweden, Switzerland and Ukraine so that it now encompasses 15 organisations in 13 countries across Europe.

Purpose, Tasks and Objectives

The purpose of SMA Europe is to provide a framework to stimulate collaboration and accelerate translational research pathways in SMA and promote patient care. Cooperation between European organisations is meant to enhance and support the existing efforts of each association by identifying areas for collaboration that are mutually advantageous to the mission of each one.

More specifically, tasks and objectives of the organisation are to increase European cooperation to achieve the following objectives:

- To improve the quality of life of people living with SMA;
- To promote the interests of people living with SMA in European health policy;
- To raise awareness of SMA with the general public, healthcare practitioners, scientists and industry;
- To identify, develop, promote and implement the best practice for SMA;
- To promote and sustain scientific and medical research in SMA;
- To increase collaboration between member countries.

SMA Europe will – inter alia - plead for the enlargement of therapeutic methods, in addition to the implementation and assurance of pan-European standardised and appropriate therapies for SMA. For this purpose, SMA Europe organises events to provide further information to stakeholders. These include regulators, pharmaceutical companies, clinicians and others.

To reach its objectives, SMA Europe works in close cooperation with other national, European and worldwide organisations, groups and institutions of similar aims and objectives and has established close contact with two American organisations in the field, Cure SMA and the SMA Foundation.

SMA Europe is supported by a Scientific Advisory Board (SAB), composed of neuroscientists and neurologists with particular expertise in spinal muscular atrophy research. The SAB advises on research grant applications and clinical trials initiatives which aim to:

- increase our understanding of the mechanisms of spinal muscular atrophy, in particular, understanding the SMN complex as it relates to the natural history of the disease;
- increase our understanding of the natural history of SMA; develop potential new therapies;
- address bottlenecks which impair rapid transition from basic research to the clinics (e.g.: Outcome measures, biomarkers, administration route of potential therapeutics).

To promote and sustain scientific and medical research in SMA
Thanks to the contribution of our member organisations, our sponsorship of research activities to date totals €4,373,184.
This has been spent on furthering the understanding of SMA (45%), the development of therapies (47%), laying the groundwork for clinical trials in Europe as well as facilitating exchanges between SMA stakeholders for the facilitation of clinical trials for SMA (8%).

Support clinical trials initiatives and patient care
SMA Europe is playing a key part in the successful introduction of clinical trials across Europe. The translation of scientific advances into effective therapies is made more difficult by issues such as the complex regulatory environment in Europe, variations in standards of care, patient enrolment, a narrow therapeutic window and a need for more sensitive biomarkers and outcome measures.

To this aim, SMA Europe has been involved in key initiatives around:

- **Mapping opportunities & challenges in developing clinical trials for SMA in Europe** through organising and funding its first international workshop, which gathered 34 scientists, clinicians and representatives of patient organisations, to establish recommendations for improving clinical trials for SMA.

- **Providing the state of the art on outcome measures & clinical readiness** through co-funding an ENMC (European Neuro-Muscular Centre) international workshop on: "Outcome Measures and Clinical Trial Readiness in Spinal Muscular Atrophy". This workshop brought together 24 researchers and industry representatives from 9 European countries, two representatives of SMA Europe (one patient, one parent of 2 SMA affected children) and one representative from SMA Foundation in the US. They met to update current knowledge on clinical trials and outcome measures for SMA. A paper was published in the Journal Neuromuscular.

- **Revising the standards of care** by co-funding an ENMC international workshop to revisit the standards of care, again: "Eight years on, revisiting the Consensus Statement of Care in SMA". This collaborative initiative between SMA Europe, SMA Support UK, Cure SMA and the SMA Foundation, gathered twenty-one clinical researchers from seven different countries (USA, UK, Italy, Germany, France, Sweden and Denmark), 2 ENMC representatives and 4 representatives of patient advocacy groups (SMA Europe, SMA Foundation and Cure SMA).

- **Determining disease impact on the general well-being of European Type II and Type III SMA patients and their therapeutic expectations** by running a survey through SMA Europe member organisations.

- **Informing the regulators about SMA** by co-hosting a one-day workshop at the European Medicines Agency (EMA), to allow SMA stakeholders to have an open forum discussion on the current challenges that face therapy development for SMA. Topics for discussion included an overview of the disease, the pharmacology of the molecules under investigation, natural history data, clinical outcome measures, the use of biomarkers in drug development and the patient perspective on clinical trials and expectations from SMA therapies.

Ongoing strategic initiatives are:
- To standardise registries across member countries
- To increase clinical trial capacity in each member country
- To build a database of SMA experts
- To ensure broad access to Spinraza™ in member countries

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Map of the venue

1. Biogen
2. Avexis
3. Novartis
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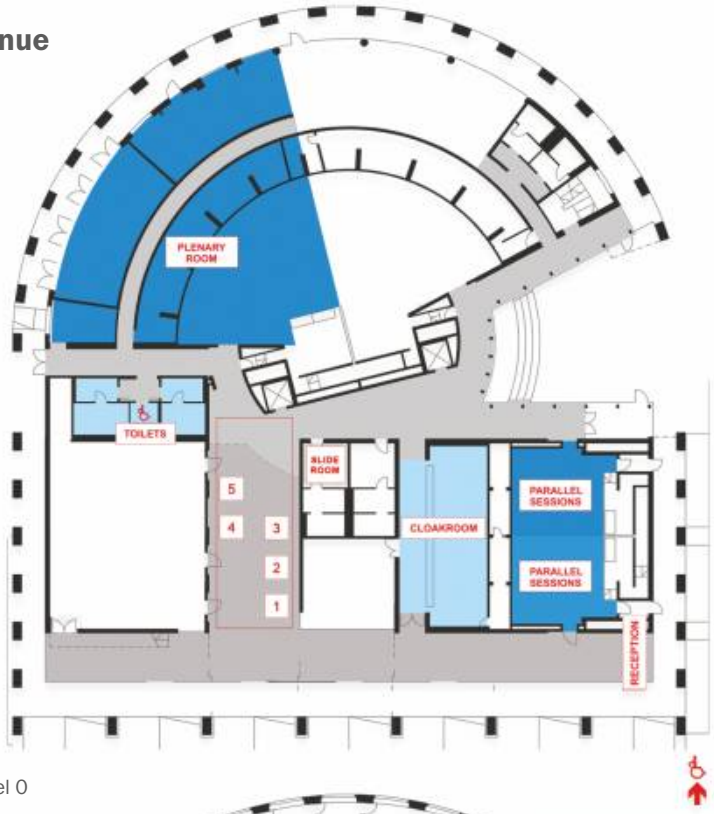
5. Medseven



Level 0

Auditorium Maximum

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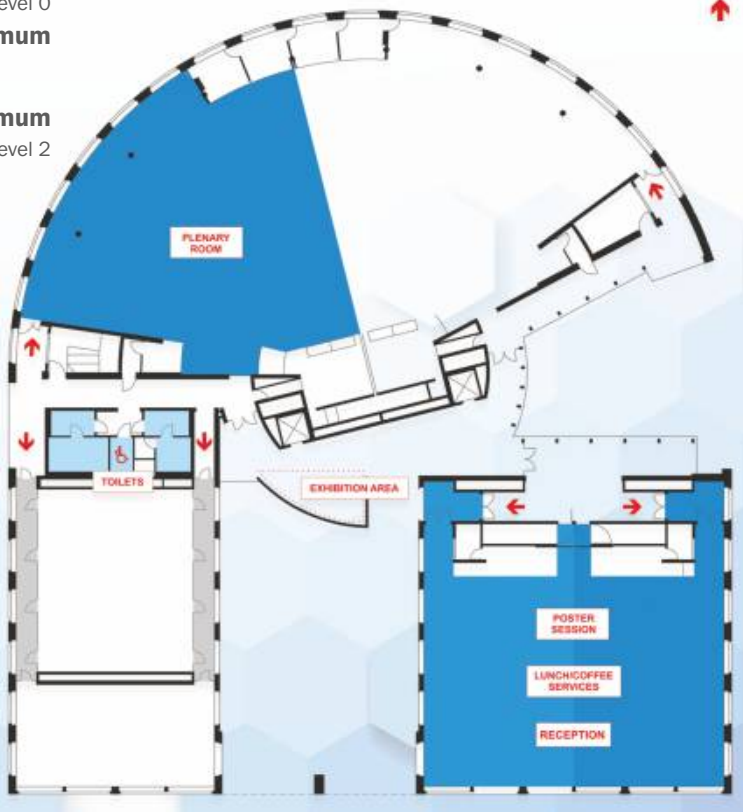

About the Scientific Committee

Chair

Brunhilde Wirth

Professor of Human Genetics and Director of the Institute of Human Genetics at the Medical Faculty of the University of Cologne, Germany.

Vice-Chair

Kevin Talbot

Professor of Motor Neuron Biology, Head of the Clinical Neurosciences Division in the Nuffield Department of Clinical Neurosciences at the University of Oxford and Honorary Consultant Neurologist at the John Radcliffe Hospital. He is Director of the Oxford Motor Neuron Disease Care and Research Centre.

Coordinator

Vanessa Christie-Brown

SMA Europe Coordinator and scientific administrator.

Members:

Enrico Bertini

Paediatric Neurologist, Head of the Unit of Neuromuscular and Neurodegenerative Disorders at the Bambino Gesu' Children's Research Hospital in Rome, and Contract Professor in Neurogenetic disorders at the School of Clinical Genetics of the Catholic University of Rome.

Serge Braun

Scientific Director of AFM (Association Française contre les Myopathies), France.

Arthur Burghes

Professor at the Center for Muscle Health & Neuromuscular Disorders, The Ohio State University/ Nationwide Children's Hospital, Columbus, Ohio, USA.

Stefania Corti

Neurologist, Associate Professor of Neurology and Principal Investigator of Neural Stem Cell Lab at the University of Milan in Italy.

Richard Finkel

Paediatric Neurologist and Division Chief of the Division of Neurology, Department of Paediatrics at Nemour's Children's Hospital in Florida, USA.

Tom Gillingwater

Professor of Neuroanatomy at the University of Edinburgh and Editor-in-Chief of the Journal of Anatomy and Academic Editor of PLoS One. He also teaches gross human anatomy and neuronanatomy to medical students and MSc students.

Cécile Martinat

Head of INSERM/UEVE UMR 861 in I-STEM, the first institute in France dedicated to the use of human pluripotent stem cells to study and treat monogenic diseases.

Laurent Servais

Child Neurologist at the Institut de Myologie in France and lead of i-Motion, a paediatric clinical trial platform for neuromuscular diseases.

Eduardo Tizzano

Paediatrician & Medical Geneticist, Director of the Department of Clinical and Molecular Genetics and Unit of Rare Diseases, Hospital Universitari Valle Hebrón, Barcelona, Spain.

Ludo van der Pol

Neurologist and Professor of Neurology, head of the Netherlands SMA center at the University Medical Center Utrecht (UMCU) in The Netherlands.

Ulrika Kreicbergs

Ulrika Kreicbergs holds a professorship in palliative care for children and youth at Ersta Sköndal University College and is also associated to the Karolinska Institutet in Stockholm, Sweden.

Podium Abstracts

Short biography of young speakers

Ewout Groen

Short CV

I obtained my degrees in Biomedical Sciences (BSc) and Neuroscience (MSc) at the University of Utrecht, the Netherlands. I then did my PhD research in the labs of Prof van den Berg and Prof Pasterkamp at the University Medical Center Utrecht, studying the genetics and molecular mechanisms that underlie amyotrophic lateral sclerosis (ALS). I then moved to the University of Edinburgh where I joined Prof Gillingwater's lab to study the cellular mechanisms of SMA, ultimately aiming to identify novel and improved therapeutic targets. I am supported by a Wellcome Trust fellowship that allows me to further develop my own research interests and line of research.

Short statement

I was born in The Hague, the Netherlands. My father is a gastroenterologist and I therefore grew up hearing a lot about medicine and I was always interested in that area of work. I was more interested in the scientific than the clinical side of medicine and therefore went on to study biomedical sciences. When I was working on ALS during my PhD, I identified possible overlapping mechanisms between ALS and SMA. I have studied SMA since and have found this very interesting; it is an extremely exciting time to be working on SMA, with many new questions and challenges arising after the initial approvement of Spinraza. I am working on a range of projects that have in common the goal to gain a better understanding of why motor neurons are the primary pathological target in SMA. I am very happy to be taking part in this congress; it is a fantastic and accessible opportunity for the wider European SMA committee to meet, exchange ideas and advance the field of SMA research. When I am not working I enjoy music in all aspects (playing the piano, collecting vinyl records, attending concerts), good food and wine, cycling and running.

Niko Hensel

Short CV

Niko Hensel studied Biochemistry in Hannover and graduated in 2010. In his diploma work he was already engaged in the SMA-field and examined altered neurotrophic signaling. He continued this work in the Claus Group during his PhD at the Center of Systems Neuroscience in Hannover expanding his interests on signaling upstream of the actin cytoskeleton. In 2015 he was a postdoctoral research fellow in Neuroinfectiology but returned to the SMA-field in 2016. He received a SMA-Europe fellowship to continue his work on altered signaling in SMA.

Short statement

"I was fascinated by the research going on in the SMA-field by the very first time I got in contact with it during my diploma work in the Claus Group. Since then I kept going on. The unique SMA-field offers a variety of perspectives of modern and exceptional biomedical research: Here, you can really see how molecular research is translated into clinics. That's why I am really looking forward to the SMA-Europe conference. To gain new insights from experts of other disciplines all dedicated to – and connected by – SMA-research."

Eva Janzen

Short CV

Eva Janzen is a PhD student in the research group of Brunhilde Wirth at the Institute of Human Genetics, Cologne, Germany, working on SMA protective modifiers. Eva started to study at the University of Cologne in 2008, receiving her B.S.C.. in Biology in 2011, followed by a M.Sc. in Biological Sciences in 2013. While, she accomplished her B.Sc. in the Cologne Center for Genomics identifying new mutations causing microcephaly, she completed her M.Sc. under the supervision of Brunhilde Wirth identifying novel Plastin3 interacting partners. Additionally, Eva completed several courses including a project management course at the Open University Hagen, Germany.

Short statement

I am from Germany. Due to my interest in Biology, I started to study Biology whereas especially Genetics draw my attention. Thus, I focused on Genetics during my M.S.C.. studies and accomplished my thesis in the Institute of Human Genetics under the supervision of Brunhilde Wirth and thereby started to work on SMA. My research aim is to identify new modifiers of SMA that could be used as potential therapeutic target. During the congress, I hope to receive feedback to my own work, to meet and get to know many SMA researchers and to gain new ideas for further studies. In my leisure time, I like to do sports especially running and riding.

Shermaine Tay

Short CV

I graduated with a bachelor degree with Honours in Life Science at the National University of Singapore in 2014. I then continued as a PhD student working with Prof. Christoph Winkler at the same university from 2015 onwards. Previously, I have also interned at the National Institute of Genetics in Japan with Prof. Koichi Kawakami as well as at the Institute of Molecular and Cell Biology in Singapore with Dr. Vladimir Korzh. So far, I have co-authored a research article “Transcriptional enhancement of Smn levels in motoneurons is crucial for proper axon morphology in zebrafish” in Scientific Reports.

Short statement

My name is Shermaine Tay and I am a third-year graduate student at the Department of Biological Sciences at the National University of Singapore (NUS). I had my first exposure to SMA research as an undergraduate attachment student working in the lab of Prof. Christoph Winkler at NUS. During this attachment, I used a zebrafish model to study the role of Schwann cell defects in SMA. This great and enjoyable experience spurred me on to undertake a graduate study in this field and pursue my research interests in SMA. Currently, I am working on two projects. First, I continue to work on Schwann cells in the zebrafish SMA model to investigate the molecular basis of noncell autonomous effects leading to motor neuron degeneration. In my second project, I am using CRISPR genome editing to establish a mutant zebrafish model for SMA by engineering a splice silencer site as found in human SMN2 into the zebrafish SMN locus. At this upcoming congress, I am eagerly looking forward to share my results with specialists in the field to collect as much valuable feedback and input as possible. I also wish to understand the current trend in SMA research, which is moving extremely fast, and of course to meet new friends and colleagues to establish collaborations and networks. Besides work, I am very much interested in Japanese culture (i.e. language, food, travel), and will be more than happy to share my experiences with anybody interested.

Laura Torres-Benito

Short CV

Laura Torres-Benito studied Biology at the University of Sevilla where she obtained the Bachelor and Master degree in Molecular Genetics and Microbial Biotechnology. In 2012, she graduated with summa cum laude at the same University, under the supervision of Prof. Lucia Tabares. As a result of her PhD she obtained, in 2015, the Extraordinary Award for Doctoral Thesis. She is currently working in Prof. Brunhilde Wirth lab, in the Institute of Human Genetics of the University of Cologne. During her postdoctoral period, she has been co-author in three highly esteemed publications and a book chapter, and holds two grants: from AFM Telethon and SMA Europe.

Short statement

I am originally from South-West of Spain. Since I was a child, I was knee on science and verycurious about how nature and everything around me was working. I moved to study at the University of Sevilla and it was during my PhD period when I started to work on SMA. After a fruitful collaboration with Prof. Brunhilde Wirth, I went to Cologne to work with her, shifting my research area from physiology to genetic. Currently, I focus my research in different projects to discover and better understand genetic modifiers in SMA and to development new combinatorial therapies. In my free time, I play piano and love climbing. I like to think that our investigation is like an upward arrow, always pointing up, like a climber, to unveiling the unsolve questions of this devastating diseasewhich is SMA.

Audrey Winkelsas

Short CV

Audrey obtained her Bachelor of Science in Biochemistry in 2015 from the University of Miami, Coral Gables, in Florida, USA. During her degree, she worked as an intern in the lab of Dr. Matthew Disney, in the department of Chemistry, at The Scripps Research Institute in Jupiter, Florida. She is now a DPhil candidate, through the NIH Oxford-Cambridge Scholars Program, at the University of Oxford, in the UK. Audrey is currently working at the National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland as a Pre-doctoral fellow, supervised by Dr. Kenneth Fischbeck and Prof. Matthew Wood.

Short statement

During a summer undergraduate research fellowship in Matthew Disney's lab at The Scripps Research Institute, RNA biology first sparked Audrey's interest. At that point, her broad desire to study SMA became focused on RNA-based therapeutics for SMA. Originally from Florida, she is currently living in Maryland where she is a graduate student in a partnership program between the National Institutes of Health and the University of Oxford. Audrey is excited for the energizing atmosphere of SMA Europe's first congress, and is looking forward to receiving feedback from others in the field. When not in the lab, Audrey has a zealous appetite for choral music and history.

Programme

Thursday
25th January 2018

13.45 **Keynote: Where have we come, where do we go?**
Arthur Burghes, PhD, Ohio State University, USA

14.30 **Session 1:**
SMN function importance in the context of this new era - Basic function in splicing: RNA-mediated mechanisms of Spinal Muscular Atrophy
Livio Pellizzoni, PhD, Columbia University, USA

15.00 **Unfolding the role of SMN protein in controlling translation in vivo: implications for Spinal Muscular Atrophy**
Gabriella Viero, PhD, Institute of Biophysics, CNR Unit at Trento, Italy

15.15 **Splicing analysis in a zebrafish model for Spinal Muscular Atrophy identifies transcripts important for motor neuron and Schwann cell function**
Shermaine Tay, BSc, National University of Singapore, Singapore

BREAK

16.00 **Session 2:**
Neuronal-specific function of SMN: Altered Axonal Actin Dynamics in Spinal Muscular Atrophy
Michael Sendtner, MD, PhD, University of Wursburg, Germany

16.30 **Converging mechanisms of p53 activation underlie selective degeneration of motor neurons in SMA**
Christian Simon, PhD, University of Leipzig, Germany

16.45 **Defining Conserved Gene Networks Affected in Spinal Muscular Atrophy using Drosophila model**
Yaka Yokokura, PhD, Okinawa Institute of Science and Technology, Japan

17.00 **Neuronal activity regulates DROSHA via autophagy in Spinal Muscular Atrophy**
Min Jeong Kye, PhD, University of Cologne, Germany

17.15 **Temporal and tissue variability of SMN protein levels in mouse models of SMA**
Ewout Groen, PhD, University of Edinburgh, UK

17.30 **POSTER SESSION A**

19.00 **Reception**

DO NOT TAPE, TAKE PICTURES, RECORD ETC

Friday
26th January 2018

08.00 **Novartis Symposium**
Serum neurofilament light chain (NfL) as a potential biomarker of Spinal Muscular Atrophy
Prof D. Leppert

09.00 **Session 3:**
SMA as a systemic disease
Charlotte Sumner, MD, PhD, John Hopkins Medical Institute, USA

09.30 **SMN around the clock: circadian dysregulation in SMA**
Melissa Bowerman, PhD, University of Keele, UK

09.45 **The development of heart defects in a mouse model of severe SMA**
Simon Parson, PhD, University of Aberdeen, UK

10.00 **Abnormal fatty acid metabolism is a feature of spinal muscular atrophy**
Rashmi Khotary, PhD, Ottawa Hospital Research Institute, Canada

10.15 **Identification and evaluation of new biomarkers for SMA – skeletal muscle and mitochondrial deficits**
Nicole Hellbach, PhD, F. Hoffmann-La Roche Ltd

BREAK

11.00 **Session 4:**
Modifiers of phenotype (Protective modifiers help to unveil the cellular mechanism and to develop combinatorial therapies in spinal muscular atrophy)
Brunhilde Wirth, PhD, University of Cologne, Germany

11.30 **CHP1 Reduction Ameliorates SMA Pathology by Restoring DNM1 Hyperphosphorylation and Endocytosis**
Eva Janzen, MSc, University of Cologne, Germany

11.45 **Improvement of synaptic transmission at the NMJ in a mouse model of Spinal Muscular Atrophy**
Rocio Tejero, PhD, University of Seville, Spain

12.00 **Comparison of independent screens on differentially vulnerable motor neurons reveals alpha-synuclein as a common modifier in motor neuron diseases**
Lyndsay Murray, University of Edinburgh, UK

12.15 **RNA-Seq and Motif Analysis of Human Motor Neurons Reveals a Critical Role of SMN/SYNCRIP complex and Motif 7 in Spinal Muscular Atrophy**
Stefania Corti, MD, PhD, University of Milan, Italy

LUNCH

13.00 **Avexis Symposium**
Gene Replacement Therapies for Monogenic Diseases, with focus on Spinal Muscular Atrophy
Dr. Samiah Al-Zaidy

14.00 **Session 5:**
Pre-clinical combined therapies; Combinatorial opportunities with splice-switching ASOs in SMA
Christian Lorson, PhD, University of Missouri, USA

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14.30 **Combinatorial ASO therapy using SMN-dependent and SMN-independent protection -NCALD reduction - against SMA**
Laura Torres-Benito, PhD, University of Cologne, Germany

14.45 **Targeting the 5'UTR of survival motor neuron 2 (SMN2) to increase its expression in a disease model of spinal muscular atrophy**
Audrey Winkelsas, BSc, National Institutes of Health, USA & University of Oxford, UK

15.00 **Dysregulated Signaling in SMA: from isolated pathway approaches to a clustered network representation**
Niko Hensel, PhD, Hannover Medical School, Hannover, Germany

15.15 **Improved in vitro models of the human blood-brain barrier (BBB) using endothelial cells derived from induced pluripotent stem cells (iPSCs) for testing CNS therapeutics**
Jamuna Selvakumaran, PhD, Royal Holloway, University of London, UK

BREAK

15.30 POSTER SESSION B

16.30 Workshops

1. **European collaboration: newborn screening; registries on treated patients; the impulse of research networks**
   Enrico Bertini, MD, PhD, Catholic University of Rome, Italy
   Eduardo Tizzano, MD, PhD, Hospital Vall d´Hebron, Spain

2. **Big data & registries**
   Hanns Lochmuller, MD, PhD, University of Newcastle, UK

17.30 Magic Trip (faculty)

19.00 Gala dinner (faculty)

Saturday
27th January 2018

08.00 Biogen Symposium
A multidisciplinary conversation on the evolving care for patients with SMA

Faculty:
Bart Bartels - Physiotherapist
Professor Katarzyna Kotulska-Józwiak - Paediatric neurologist
Professor Francesco Muntoni - Paediatric neurologist
Inge Schwersenz - Patient advocacy group representative

Moderator:
Vivienne Parry

09.00 Session 6:
Emerging phenotypes & SOCs
Laurent Servais, MD, PhD, i-Motion, Institut de Myologie, France

09.30 **MRI of the cervical spinal cord and nerve roots in SMA**
Marloes Stam, MD, University of Utrecht, The Netherlands

DO NOT TAPE, TAKE PICTURES, RECORD ETC

09.45 **End of Study Results from ENDEAR: Proportions of HINE-2 and CHOP INTEND Responders**
Eduardo Tizzano, MD, PhD, Hospital Vall d´Hebron, Spain

10.00 **Cognitive Development, Language and use of Augmentative Alternative Communication in SMA1 Children in Italy**
Grazia Zappa, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

10.15 **Clinical challenges in the treatment of spinal muscular atrophy (SMA) with Nusinersen**
Claudia Wurster, MD, University of Ulm, Germany

BREAK

11.00 **Session 7:**
Challenges of clinical trials & beyond (& Benefits of Earlier Treatment With Nusinersen in Infants and Children With Spinal Muscular Atrophy)
Richard Finkel, MD, Nemours Children's Hospital, Florida, USA

11.30 **Clinical effects of nusinersen injections in SMA type 1 patients older than 7 months: 10 months of follow up**
Karolina Aragon-Gawinska, MD, i-Motion, Institut de Myologie, France

11.45 **More than just fun and games: ACTIVE workspace volume video game quantifies upper extremity function in individuals with spinal muscular atrophy (SMA)**
Linda Lowes, PT, PhD, Nationwide Children's Hospital, Columbus, USA

12.00 **FIREFISH, a multi-center, open-label trial to investigate the safety and efficacy of RG7916 in babies with Type 1 SMA: Study update and real-life experience of study implementation**
Giovanni Baranello, MD, Carlo Besta Neurological Research Institute Foundation, Milan, Italy

12.15 **A long-term, open-label follow-up study of olesoxime in patients with Type 2 or non-ambulatory Type 3 spinal muscular atrophy who participated in a placebo-controlled Phase 2 trial**
Francesco Muntoni, MD, PhD, University College London, UK

12.30 **Conclusions**
Francesco Muntoni, University College London, UK

13.00 **Awards & closing ceremony**

LUNCH

14.30 **Congress ends**

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Podium abstracts

SESSION 1 - SMN FUNCTION IMPORTANCE IN THE CONTEXT OF THIS NEW ERA - BASIC FUNCTION IN SPLICING

O1: UNFOLDING THE ROLE OF SMN PROTEIN IN CONTROLLING TRANSLATION IN VIVO: IMPLICATIONS FOR SPINAL MUSCULAR ATROPHY

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Spinal Muscular Atrophy (SMA) is an autosomal recessive disease caused by low levels of SMN protein. SMN undertakes various roles in the cytoplasm and co-sediments with polysomes, but the contribution of SMN/polysome-crosstalk to disease pathogenesis has never been established.

To understand the role of SMN in translation, we investigated the consequences of low levels of SMN for translational regulation in vivo, using an established mouse model of SMA. We report widespread and robust perturbations in translation efficiency and mRNA recruitment on polysomes in pathologically relevant tissues at both early and late symptomatic stages. In parallel we show that decreased translation efficiencies are associated with SMA disease progression. Sequencing of mRNAs recruited on polysomes (POL-Seq) and ribosome protected fragments (RIBO-Seq), led to the identification of RNAs that are differentially associated with polysomes in SMA, identifying ribosome biology and translation as central processes affected by SMN depletion. This was further supported by a decrease in the number of ribosomes in SMA motor neurons in vivo, suggesting that ribosome biology is likely an important, yet largely neglected factor in motor neuron degeneration. Polysome perturbations and subsequent translational defects were rescued by treatment with an antisense oligonucleotide (ASO) restoring SMN levels, indicating that these defects are both reversible and eventually amenable to therapeutic intervention.

Finally, by newly developed strategies to dissect at nucleotide resolution the position of ribosomes on polysomes (Active-RiboSeq and SMN-specific ribosome protected fragments), we unravel intriguing new scenarios for mechanistic explanation of translational defects in SMA.

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O2P: SPLICING ANALYSIS IN A ZEBRAFISH MODEL FOR SPINAL MUSCULAR ATROPHY IDENTIFIES TRANSCRIPTS IMPORTANT FOR MOTOR NEURON AND SCHWANN CELL FUNCTION

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It remains debated how deficiencies in the ubiquitously expressed Survival Motor Neuron (SMN) gene result in the degeneration of motor neuron (MNs) in Spinal Muscular Atrophy (SMA). One hypothesis suggests that MNs are selectively vulnerable to deficient pre-mRNA splicing, and that defects in associated Schwann cells contribute to MN degeneration in a non-cell autonomous manner. To identify aberrantly spliced transcripts that are crucial for survival and activity of motor neurons, our lab performed transcriptome analyses in a zebrafish SMA model. RNAseq analysis of SMN deficient FAC-sorted motor neurons and Schwann cells revealed 284 and 767 significant alternative splicing events, respectively. Among these events, we identified aberrantly spliced transcripts for srsf6b, which encodes a tissue-specific splice factor enriched in the ventral spinal cord. We generated zebrafish srsf6b mutants and found that they display severe motor axon branching defects. This suggests that srsf6b is essential for MN function and possibly amplifies the consequences of a general SMN-induced splicing defect particularly in MNs. Our transcriptome analysis also identified neurexin2a (nrxn2a) as a novel downstream target of SMN. Reduced SMN levels increase exon skipping in nrxn2a in both zebrafish and mouse SMA models. Maternal-zygotic nrxn2a zebrafish mutants exhibit early motor axonal pathfinding and synaptogenesis defects, which later develop into muscular atrophy at adult stages. Zygotic nrxn2a mutants show normal axon pathfinding but interestingly exhibit increased anxiety behavior in adulthood. Finally, we also obtained evidence for a non-cell autonomous contribution to MN degeneration. Using live calcium imaging in the transparent zebrafish embryos, we found that a SMN deficiency causes reduced excitability of Schwann cells. Importantly, transgenic restoration of SMN specifically in Schwann cells alleviated motor axonal pathfinding defects. Our preliminary data suggest that a novel candidate gene, which is down-regulated in SMN-deficient Schwann cells, contributes to MN degeneration in the zebrafish SMA model. Together, our findings provide further support for the hypothesis that defects in pre-mRNA splicing and non-cell autonomous contributions of Schwann cells are critical in SMA pathology. This project is supported by the National Medical Research Council (NMRC; CBRG/0046/2013), Singapore.

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SESSION 2 - NEURONAL-SPECIFIC FUNCTION OF SMN

O3: CONVERGING MECHANISMS OF P53 ACTIVATION UNDERLIE SELECTIVE DEGENERATION OF MOTOR NEURONS IN SMA

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The hallmark of spinal muscular atrophy (SMA) – an inherited disease caused by ubiquitous deficiency in the SMN protein – is the selective, cell autonomous degeneration of subsets of spinal motor neurons. However, the basis for the differential vulnerability of distinct motor neuron pools in SMA is unknown.

To investigate potential pathways mediating motor neuron degeneration, we performed comparative gene expression profiling of vulnerable and resistant motor neurons from a severe mouse model of SMA prior to the onset of neuronal death. This analysis identified the upregulation of p53 transcriptional targets specifically in vulnerable motor neurons, providing evidence for p53 pathway activation. Analysis by immunohistochemistry revealed strong and selective nuclear accumulation of p53 at early pre-symptomatic stages in vulnerable motor neurons of SMA mice. Remarkably, inhibition of p53 either pharmacologically or genetically by AAV9-mediated RNAi knockdown resulted in near complete rescue of motor neuron death in SMA mice, establishing that motor neuron degeneration is p53-dependent. At late disease stages, however, nuclear accumulation of p53 extends to resistant motor neurons and spinal interneurons but is not associated with cell death. To identify potential distinguishing features of p53 activation selectively in vulnerable SMA motor neurons, we analyzed post-translational modifications of p53 that play a fundamental regulatory role on its function. Strikingly, we found a specific modification of p53 present exclusively in vulnerable motor neurons of SMA mice that correlates precisely with the timing of motor neuron degeneration.

In this work, we establish p53 as a major driver of motor neuron death in SMA. We further demonstrate that while p53 activation occurs earliest in vulnerable motor neuron populations, later progresses in resistant motor neurons as well as other spinal neurons that do not degenerate in the disease. Thus, nuclear accumulation of p53 is necessary but not sufficient to trigger neuronal death. Importantly, we identified a post-translational modification of p53 that exclusively marks vulnerable SMA motor neurons at the onset of the degenerative process. Taken together, our findings reveal a mechanism where two distinct events induced by SMN deficiency - nuclear accumulation and specific post-translational modification of p53 – converge in vulnerable motor neurons to cause their selective degeneration in SMA.

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04P: DEFINING CONSERVED GENE NETWORKS AFFECTED IN SPINAL MUSCULAR ATROPHY USING DROSOPHILA MODEL

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Spinal Muscular Atrophy (SMA) is a devastating inherited disorder characterized by progressive loss of motor activity due to death of motor neurons, structural and functional failure of neuromuscular synapses and muscle atrophy. The cause of SMA is primarily reduction of Survival Motor Neuron (SMN) protein in spinal cord motor neuron due to mutation of SMN1 gene, so restoring SMN protein levels in spinal cord motor neuron has been primary target of therapeutic approaches. However, underlying mechanism that manifest SMA pathological phenotypes are not well understood. Furthermore, gene networks that link low levels of SMN expression and SMA pathologies is remained poorly understood. We have sought to address this extant question by using Drosophila SMA model. Elimination of Smn in Drosophila ovary by germline recombination resulted in developmental arrest at the early stages of oogenesis. Homozygous Smn deletion mutant survives through embryo genesis but dies during early larval stages as diminishing maternal Smn. Zygotic knockdown of Smn using RNA interference exhibited phenotypes similar to pathological phenotypes seen in human SMA patients: such as loss of motor axons, degeneration of muscles and defects in the neuromuscular junction. Moreover, the severity of mutant phenotype depends on Smn protein dose. Our genetic screens revealed over 300 genes may functionally interact with Smn. Further analysis focused on two canonical pathways, BMP and FGF signaling pathways, indicated that modulation of trans-synaptic signaling can rescue the Smn defects. As we found that FGF and BMP pathways modulate distinct aspects of SMA motor neuron and NMJ pathologies, we examined if reduction SMN levels alter expression of any known components of those pathways by quantitative PCR assay targeting isoforms of 16 components of those pathways and found that expression of only one or two transcriptional isoforms of a few genes were altered. As SMN protein is a core component of the Gemin complex and support biogenesis of RNA processing machinery, we have been examining the impact of Smn mutations on global gene expression and splicing patterns in CNS of Drosophila SMN mutant by transcriptome analysis. In addition, we are analyzing and comparing those data with RNAseq data that we obtained from SMA patient fibrobrast driven motor neuron cells to defining conserved pathway between human SMA and Drosophila SMA model.

NOTES

O5P: NEURONAL ACTIVITY REGULATES DROSHA VIA AUTOPHAGY IN SPINAL MUSCULAR ATROPHY

Inês do Carmo Gonçalves[1], Johanna Brecht[1], Maximilian Thelen[1], Wiebke Rehorst[1], Miriam Peters[1], Laura Torres-Benito[1], Darius Ebrahimi-Fakhari[2], Natalia Kononenko[3], Mustafa Sahin[2], Brunhilde Wirth[1], **Min Jeong Kye[1]**

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Dysregulated miRNA expression and mutation of genes involved in miRNA biogenesis have been reported in motor neuron diseases including spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS). Therefore, identifying molecular mechanisms governing miRNA expression is important to understand these diseases. Here, we report that expression of DROSHA, which is a critical enzyme in the microprocessor complex and essential for miRNA biogenesis, is reduced in motor neurons from a SMA mouse model. DROSHA proteins are degraded by neuronal activity induced autophagy machinery, which is also abnormally regulated in SMA. Blocking neuronal activity or the autophagy-lysosome pathway restores DROSHA levels in SMA motor neurons. Moreover, reducing DROSHA levels enhances axonal growth. As impaired axonal growth is a well described phenotype of SMA motor neurons, these data suggest that DROSHA reduction by autophagy may mitigate the phenotype of SMA. In summary, these findings suggest that autophagy regulates RNA metabolism and neuronal growth via the DROSHA/miRNA pathway as a compensatory mechanism in SMA.

NOTES

O6P: TEMPORAL AND TISSUE VARIABILITY OF SMN PROTEIN LEVELS IN MOUSE MODELS OF SMA

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Spinal muscular atrophy is caused by deleterious variants in SMN1 that lead to a loss of full-length SMN protein expression. A second, almost identical SMN gene, called SMN2, is alternatively spliced, leading to truncated mRNA, the protein product of which is unstable and quickly degraded. A minority (~10%) of SMN2-derived mRNA, however, contains the full SMN sequence and is translated into stable, full-length SMN protein. Previous studies have shown that SMN expression requirements vary during development. Moreover, SMN expression is required in all tissues, but as SMA is primarily a motor neuron disease tissue requirements might vary. Surprisingly, little is known about the amount of full-length SMN protein that is derived from SMN2 mRNA and how protein expression varies over time and between tissues. In this study, we aimed to address this issue by determining absolute levels of SMN protein expression in 6 healthy control mice tissues (brain, spinal cord, muscle [m. gastrocnemius], heart, liver and kidney) at 3 developmental time points, matching pre-, early- and late-symptomatic stages of disease in mouse models of SMA. Next, we compared the relative amounts of SMN in healthy controls to that of affected littermates in 2 different mouse models of SMA ('Taiwanese' and SMN2B) in the same set of tissues. We found that, in control tissue, the absolute amount of SMN varies greatly over time and across tissues. Moreover, the level of SMN depletion in SMA models varies between the tissues investigated and between the 2 different models. These results suggest that SMN requirements likely correlate with the severity in which tissues are affected. Moreover, our results suggest that, despite a constant genetic background in all tissues, cellular mechanisms must be in place that lead to considerable variability in SMN protein levels based on developmental stage and tissue requirements. Studying the pathways that regulate the variability in SMN expression is likely to provide interesting new insights into SMA pathogenesis. Also, our findings have implications when designing and interpreting results from experimental as well as therapeutic studies.

NOTES

SESSION 3 - SMA AS A SYSTEMIC DISEASE

O7: SMN AROUND THE CLOCK: CIRCADIAN DYSREGULATION IN SMA

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Behaviour and physiology are regulated by a 24 hr circadian rhythm governed by core clock genes and a functional relationship exists between circadian rhythms and metabolic homeostasis. Spinal muscular atrophy (SMA) is a neuromuscular disorder caused by loss of the survival motor neuron (SMN) protein and characterised by motor neuron loss and muscle atrophy. Tissues outside the CNS such as heart, pancreas, liver and muscle display intrinsic defects in SMA and these organs influence metabolic health and are regulated by internal clocks. Seeing as several metabolic and sleep perturbations have been reported in SMA animal models and patients, we set out to investigate circadian rhythms in a severe SMA mouse model. Various metabolic tissues (tibialis anterior, liver, heart, white adipose tissue and brown adipose tissue) and spinal cord were harvested from P2 (pre-symptomatic) and P7 (symptomatic) Smn-/-;SMN2 and Smn+/-;SMN2 healthy littermates every 4 hrs over a 24 hr time course. We uncover for the first time that the Smn gene displays a diurnal expression in various metabolic tissues during early development. Further, we demonstrate a disruption of the diurnal rhythmicity of the core clock genes and clock output genes in SMA metabolic tissues during disease progression. Importantly, we find that a controlled light exposure attenuates the SMA phenotype at both phenotypic and molecular levels. Our study gives further evidence that SMA is a multi-system disease and indicates that loss of the SMN protein has a systemic effect on circadian metabolism, which could have significant implications for the development of peripheral therapeutic approaches and overall clinical care management of patients.

NOTES

08P: THE DEVELOPMENT OF HEART DEFECTS IN A MOUSE MODEL OF SEVERE SMA

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Heart defects are consistently described in patients and animal models of Spinal Muscular Atrophy (SMA), but we have no clear understanding of their aetiology. Here, we have carried out a systematic and detailed morphological assessment of heart pathology in the Taiwanese mouse model of severe SMA, focusing on events prior to the appearance of overt neuromuscular symptoms. Hearts are grossly normal and their weight relative to body weight is not significantly different between SMA and Het control mice from P1 (birth) to P8 (late symptomatic) timepoints. However, gross histological differences are apparent as ventricles are significantly enlarged with a thin interventricular septum at P3 and P5, while left ventricular wall is thinner at P1, P3 and P5 in SMA compared to control mice.

Immunohistochemistry revealed that ordering of the cardiomyocytes making up the heart walls is altered: first, the tri-laminar, spiral arrangement of cardiomyocytes essential for efficient ventricular contraction and emptying is not apparent in SMA, rather an embryonic circumferential arrangement is present. This apparent developmental delay is preceded by a decrease in expression of essential basement membrane protein collagen IV at P1; second, the embryonic trabecular structure of the ventricular luminal surface persists until P5 in SMA but not control hearts. Together, these defects describe a pattern of delayed development, as seen in other organs.

We found no differences in cell proliferation between P1 and P5, but significantly elevated cell death at P5, which was preceded by evidence of oxidative stress at P3 and P5, suggesting that degenerative changes my become important at later stages of development.

Significantly more blood is found pooled in SMA hearts in this fixed but non-perfused state, particularly in the ventricles between P1 and P5. This suggests that the defects reported in the heart wall are associated with decreased functionality, resulting in incomplete ventricular emptying. Taken together with previously described widespread defects in capillary beds and altered red blood cell and platelet production, a cardiovascular system-wide pathology is indicated in SMA.

These systemic pathologies are likely to become increasingly apparent in children treated with Spinraza (Nusinersen), where neuromuscular symptoms are alleviated and life extended, therefore combinatorial therapies to address them must be developed.

NOTES

09: ABNORMAL FATTY ACID METABOLISM IS A FEATURE OF SPINAL MUSCULAR ATROPHY

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Spinal muscular atrophy (SMA) is a devastating neuromuscular disorder characterized by paralysis and muscle weakness. Recent evidence suggests that SMA is a multi-organ disease. We have previously shown abnormal glucose homeostasis due to altered pancreatic islet cell distribution in SMA mouse models and in human patients. Here, we have assessed whether liver and/or skeletal muscle lipid metabolic defects are present in SMA. A thorough characterization of gross, histological and molecular changes in the liver and skeletal muscle from SMA model mice was performed. Progressively paler livers were noted in Smn2B/- mice as they aged, a possible sign of fatty liver. Haematoxylin and eosin staining showed a dramatic decrease in cellular density in P19 Smn2B/- livers, potentially because of fatty infiltration. Fatty acid quantification and profiling highlighted a 25-fold increase in triglycerides, a 6-fold increase in cholesterol esters, and misregulation of all other lipids classes, albeit to a milder extent. Of note, fatty acid profiles in each lipid class were altered. Microarray analysis of fatty acid metabolism pathways revealed major changes in mRNA of both liver and muscle of symptomatic mice. About 50% of the 84 genes analysed were misregulated, 25 of which were changed in both muscle and liver, while 15 and 17 changes were specific to the muscle and the liver, respectively. Taken together, these results provide further evidence in metabolic defects in SMA. Further investigation will be required to establish the primary mechanism of these lipid metabolic defects and understand whether they lead to additional co-morbidities in SMA patients.

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O10P: IDENTIFICATION AND EVALUATION OF NEW BIOMARKERS FOR SMA – SKELETAL MUSCLE AND MITOCHONDRIAL DEFICITS

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Spinal muscular atrophy (SMA) is a severe genetic disorder that manifests in progressive neuromuscular degeneration. SMA originates from loss-of-function mutations of the SMN1 (survival motor neuron 1) gene. Recent evidence suggests that in SMA not only denervation, but also skeletal muscle deficits and mitochondrial dysfunction significantly contribute to the progression of disease.

In this study, we explore potential biomarkers to measure peripheral deficiencies in SMA patients. We aim to characterize skeletal muscular dysfunctions and mitochondrial deficits in SMA patients in more detail and to identify potential biomarkers quantifying these deficits. Discovery of novel exploratory biomarkers determining skeletal muscular and mitochondrial dysfunctions may allow better assessment of potential therapeutic benefit of investigational medicines like splicing modifiers and mitochondrial protecting compounds on these deficits in clinical trials.

We identified significant differences in five new skeletal muscle biomarkers (GDF-8, sTnI, Fabp3, Myl3, Ckm) in plasma samples from SMA Type III patients compared to age-matched healthy volunteers. These five skeletal muscle biomarkers were compared to the standard clinical chemistry measurements ALT, AST and CK. Correlation analysis of the five skeletal biomarkers with quantitative muscle MRI and motor function measurements (MFM and 6MWT) revealed a significant correlation of GDF-8 with these parameters. An additional analysis of mitochondrial parameters in blood samples of over 100 SMA patients identified reduced circulating mitochondrial DNA levels in SMA Type II and Type III patients.

Our biomarker data suggest that five new skeletal muscle biomarkers (GDF-8, sTnI, Fabp3, Myl3, CKm) and mitochondrial DNA provide additional sensitivity and specificity to standard clinical chemistry measurements, and have the potential to become new exploratory biomarkers to monitor the skeletal muscular and mitochondrial deficits in SMA patients.

NOTES

SESSION 4 - MODIFIERS OF PHENOTYPE

O11: PROTECTIVE MODIFIERS HELP TO UNVEIL THE CELLULAR MECHANISM AND TO DEVELOP COMBINATORIAL THERAPIES IN SPINAL MUSCULAR ATROPHY

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Spinal muscular atrophy (SMA), a devastating neuromuscular disorder, affects around 1:6000 people, every 1:35 is carrier in Europe and it is the most frequent genetic cause of infant death. Recently, the first SMA therapy based on antisense oligonucleotides, namely SPINRAZA, has been FDA- and EMA-approved. SPINRAZA restores the suboptimal full-length SMN2 transcript expression and elevates SMN protein level. SMN is crucial for all cells but particularly for motor neurons and neuromuscular junctions (NMJ). In the most severe type I - accounting for 60% of SMA-affected individuals, who carry only two SMN2 copies - the elevated SMN level may be still insufficient to restore motor neuron function lifelong. We show that genetic SMA modifiers might provide additional functional support at MN and NMJ level.
Here I will talk about two human SMA protective modifier, identified in asymptomatic SMN1-deleted individuals carrying either 3 or 4 SMN2 copies. Plastin 3 (PLS3), an F-actin binding and bundling protein rescues SMA by overexpression and Neurocalcin delta (NCALD), a neuronal calcium sensor protein counteracts SMA by suppression. We found that both, PLS3 overexpression or NCALD suppression protect against SMA across species including zebrafish and mice. Moreover, both modifiers show a rescuing effect using combinatorial therapies – low dose SMN-ASO and PLS3 overexpression or NCALD suppression - in severely-affected SMA mice. Lastly, both modifiers hinted us towards the main cellular mechanism in SMA, which we believe is impaired endocytosis, and which is restored by both modifiers.

NOTES

012P: CHP1 REDUCTION AMELIORATES SMA PATHOLOGY BY RESTORING DNM1 HYPERPHOSPHORYLATION AND ENDOCYTOSIS

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Recently, the first SMA drug based on antisense oligonucleotides (ASOs) correcting SMN2 splicing, namely SPINRAZA, has been approved. However, in type I SMA patients the ASO induced elevation of SMN levels may be still insufficient to restore motor neuron (MN) function lifelong. PLS3 and NCALD are two SMN-independent protective modifiers identified in humans and proven to be effective across various SMA animal models. Both, PLS3 overexpression and NCALD downregulation protect against SMA by restoring impaired endocytosis. Nevertheless, the exact mechanism behind this protection is largely unknown.

Here, we identified Calcineurin-like EF-hand protein 1 (CHP1) as a novel PLS3 interacting protein using a yeast-two-hybrid screen. Co-immunoprecipitation and pull-down assays confirmed a direct interaction between CHP1 and PLS3. Although CHP1 is ubiquitously expressed, it is particularly abundant in neurons and specifically present at SMA relevant sites such as MN growth cones and neuromuscular junctions (NMJ). Strikingly, we found elevated CHP1 expression in SMA. Indeed CHP1 downregulation restored axonal outgrowth defects in Smn-depleted NSC34 MN-like cells, SMA zebrafish and murine SMA MNs. Most importantly, subcutaneous injection of a low-dose SMN-ASO in presymptomatic mice doubled the survival of a severely-affected SMA model, whereas additional CHP1 reduction, by a heterozygous Chp1 mutant allele, prolonged survival span by 3.2-fold. Moreover, CHP1 reduction ameliorated electrophysiological defects, NMJ size, NMJ maturation and muscle fibre size compared to low-dose SMN ASO alone. In MN-like cells, Chp1 knockdown almost tripled bulk endocytosis while clathrin-mediated endocytosis remained unaffected. Importantly, CHP1 knockdown restored bulk endocytosis in Smn-depleted cells by elevating Calcineurin phosphatase activity. As CHP1 is the counter-player of Calcineurin which collectively dephosphorylates proteins involved in endocytosis, therefore, it is crucial for synaptic vesicle endocytosis. Congruently, we found marked hyperphosphorylation of Dynamin 1 (DNM1) in SMA MNs, which was restored to control level by the heterozygous Chp1 mutant allele.

Taken together, we show that CHP1 is a novel modifier of SMA pathology that directly interacts with PLS3, and ameliorates SMA phenotypes by improving impaired endocytosis. Most importantly, we demonstrate that CHP1 downregulation is a promising SMN-independent therapeutic target for a combinatorial SMA therapy.

NOTES

013: IMPROVEMENT OF SYNAPTIC TRANSMISSION AT THE NMJ IN A MOUSE MODEL OF SPINAL MUSCULAR ATROPHY

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Spinal Muscular Atrophy (SMA), the most frequent genetic cause of infant mortality, is an autosomal recessive neurodegenerative disease characterized by the loss of α-motoneurons in the spinal cord, muscle weakness, and progressive paralysis. It is caused by the loss or mutation of the Survival Motor Neuron 1 (SMN1) gene. In SMA mouse models, synaptic transmission at the NMJ is severely impaired.

Recently, we studied possible molecular alterations responsible for the reduction in neurotransmitter release in SMA mouse models. Our work revealed a significant reduction in P/Q-type voltage-dependent calcium channels, SV2B and Syt2 proteins in SMA nerve terminals compared to controls. In agreement with these findings, binomial analysis of neurotransmitter release indicated that the decrease in the number of quanta discharged per action potential (quantal content, m) is due to a reduction in the number of functional release sites (n) in SMA motor nerve terminals. It is unknown, however, to what extent the homeostatic plasticity of SMA nerve terminals is limited by these and other alterations. To address this question we investigated the effect of two types of compounds that are known to positively modulate neurotransmission in control synapses: phorbol ester, and L-type calcium channel blockers.

We found that phorbol 12,13-dibutyrate (PDBu),a DAG analog that activates Munc13 and PKC,increased synchronous neurotransmitter release in both control (189±19%) and in SMA (153±27%) nerve terminals by increasing the number of vesicles available at release sites at rest. We also studied the effect of L-type calcium channels antagonists on secretion and found that nifedipine increased evoked release both in controls (197±20%) and in SMA (213±27%) nerve terminals. We are now investigating the possible implication of the phosphatase 2A/calmodulin kinase II (PP2A/CaMKII) system in the modulation of synaptic transmission by L-type calcium channels antagonists.

Together, our results revealed that SMN-deficient motor nerve terminals, despite their structural and functional alterations, are still able to regulate neurotransmitter release positively through different pathways.

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NOTES

014: COMPARISON OF INDEPENDENT SCREENS ON DIFFERENTIALLY VULNERABLE MOTOR NEURONS REVEALS ALPHA-SYNUCLEIN AS A COMMON MODIFIER IN MOTOR NEURON DISEASES

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In SMA, as well as in other motor neuron diseases, not all motor neuron populations are equally vulnerable. In both patients and animal models of these diseases, some motor neurons are lost very early in disease, others remain comparatively intact, even at late stages. This creates a valuable system to investigate the factors that regulate motor neuron vulnerability. In this study, we aimed to use this experimental paradigm to identify potential transcriptional modifiers. We have compared the transcriptome of motor neurons from wild-type mice, which are differentially vulnerable in the childhood motor neuron disease Spinal Muscular Atrophy (SMA), and have identified 910 transcriptional changes. We have compared this data set with published microarray data sets on other differentially vulnerable motor neurons, which were predominantly differentially vulnerable in the adult onset motor neuron disease Amyotrophic Lateral Sclerosis (ALS), and were performed in neurologically normal human, rat and mouse. This cross species and cross disease comparison has generated a refined list of differentially expressed genes, including CELF5, Col5a2, PGEMN1, SNCA, Stmn1 and HOXa5, alongside a further enrichment for synaptic and axonal transcripts. As an in vivo validation, we demonstrate that the manipulation of a significant number of these transcripts can modify the neurodegenerative phenotype observed in a Drosophila line carrying an ALS causing mutation. Finally, we demonstrate that vector-mediated expression of alpha-synuclein (SNCA), a transcript decreased in selectively vulnerable motor neurons in all four screens, can extend life span, increase weight and decrease neuromuscular junction pathology in a mouse model of SMA. In summary, we have combined multiple data sets to identify transcripts, which are strong candidates for being phenotypic modifiers, and demonstrated SNCA is a modifier of pathology in motor neuron disease.

NOTES

O15P: RNA-SEQ AND MOTIF ANALYSIS OF HUMAN MOTOR NEURONS REVEALS A CRITICAL ROLE OF SMN/SYNCRIP COMPLEX AND MOTIF 7 IN SPINAL MUSCULAR ATROPHY

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Spinal muscular atrophy (SMA) is a genetic motor neuron disease caused by the reduction of the survival motor neuron (SMN) protein, essential for pre-mRNA processing. The exact pathogenic mechanisms and the reasons of selective motor neurons (MNs) vulnerability are still not completely understood. It could be hypothesized that MNs hold specific features that make them more sensitive to lower amounts of SMN protein. SMN has been demonstrated to possess a key role in RNA splicing, although a thorough analysis of the correlation between SMN defect, specific gene expression/splicing alterations, and the presence of common RNA motif sequences in deregulated genes in human SMA MNs is still lacking.

To address this question, we performed deep RNA sequencing and bioinformatic analyses on MNs derived from induced pluripotent stem cells (iPSCs) of SMA patient and healthy subjects. We detected SMA-specific molecular changes in MNs, including abnormalities in axonal and synaptic genes such as in Neurexin and Synaptotagmin genes families. Motif enrichment analysis of differentially expressed/spliced genes in SMA-MNs revealed the presence of common sequences for RNA binding proteins. Among these, hnRNPQ (SYNCRIP), that interacts with SMN but only full-length isoform, binds several of these key MN genes suggesting that the impairment of SMN(full-length)/hnRNPQ interaction in SMA can be responsible for the observed gene expression/splicing misregulation. Overexpression of hnRPNQ in human SMA iPSCs-derived lines improved MN survival and increased motor axon length, restoring identified candidate downstream targets of RNA-processing dysfunction induced by SMN deficiency.

These data demonstrated that SMN is essential for mRNA processing in association with hnRNPs and identified selective RNA pathways disrupted in SMA that can represent alternative therapeutic targets beside SMN.

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NOTES

SESSION 5 - PRE-CLINICAL COMBINED THERAPIES

029: COMBINATORIAL OPPORTUNITIES WITH SPLICE-SWITCHING ASOS IN SMA

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Recently, several compounds designed to increase SMN protein have entered clinical trials, including antisense oligonucleotides (ASOs), traditional small molecules, and gene therapy. Expanding beyond SMN-centric therapeutics is important as it is likely that the breadth of the patient spectrum and the inherent complexity of the disease will be difficult to address with a single therapeutic strategy. Several SMN-independent pathways that could impinge upon the SMA phenotype have been examined with varied success. To identify disease-modifying pathways that could serve as stand-alone therapeutic targets, or in combination with an SMN-inducing compound, we investigated Adeno Associated Virus (AAV)-mediated gene therapy using Plastin-3 (PLS3). Here we report that AAV9-PLS3 extends survival in an intermediate model of SMA mice as well as in a pharmacologically induced model of SMA using a splice-switching ASO that increases SMN production. PLS3 co-administration improves the phenotype beyond the ASO-alone cohort, demonstrating the potential utility of combinatorial therapeutics in SMA that target SMN-independent and SMN-dependent pathways. Additionally, the ASO has been used in combination with an FDA approved small molecule, and when used in combination, leads to enhanced efficacy in SMA mice. Collectively this work highlights the potential therapeutic combinatorial opportunities that exists within SMA.

NOTES

016P: COMBINATORIAL ASO THERAPY USING SMN-DEPENDENT AND SMN-INDEPENDENT PROTECTION - NCALD REDUCTION - AGAINST SMA

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Spinal muscular atrophy (SMA) is a neuromuscular disease with an incidence of 1:6000 to 1:10,000 and the most frequent genetic cause of childhood lethality. Recently, SPINRAZA, an antisense oligonucleotide (ASO) that corrects SMN2 splicing and thus increases full-length SMN protein has been FDA- and EMA-approved for SMA therapy. However, the administration of ASOs in very severe and/or post-symptomatic patients might be insufficient to counteract the disease. We believe that additional SMN-independent therapies may be required especially in type I SMA, where only two SMN2 copies are available; and/or in all types of SMA to support motorneurons (MN) and neuromuscular junction (NMJ) function after disease onset. We identified PLS3 and NCALD as protective modifiers of SMA in humans, while their modifying role has been proven in various animal models of SMA including worm, flies, zebrafish and mice. Despite PLS3 overexpression is the stronger protective modifier, NCALD downregulation seems easier to be targeted by ASOs and to be used in combination with SMN ASOs.

Based on the encouraging results from genetically modified mice, we developed in collaboration with IONIS Pharmaceuticals Ncald ASOs to reduce NCALD level. From the initial 30 Ncald ASOs, which were generated and tested in cells and adult mice, we chose the three most efficient ones to be tested in neonatal mice. We optimized them for tolerability and efficiency in the Taiwanese SMA mouse model on mixed background (50%C57BL6/N; 50%FVB/N) developed in our lab. Ncald3 ASO showed the optimal viability, with non-toxic effects and the best decrease of the protein expression: 75% in brain and 80% in spinal cord.

To analyse the impact of reduced NCALD on the SMA phenotype, we next performed a preclinical study using presymptomatic injection of low-dose SMN+Ncald ASOs compared to SMN+control ASOs, where Ncald- or control ASOs were injected ICV at P2 and SMN ASOs subcutaneously at P1. Preliminary results showed a significant increase in CMAP (compound muscle action potential) in SMA mice injected with SMN+Ncald ASOs compared to SMN+control ASOs at P21. A future perspective of the present project is to validate the combinatorial therapy at different ages (3 and 6 months) and in postsymptomatic stages and to analyse these mice in detail (motoric abilities, MN, NMJ and muscle development).

NOTES

017P: TARGETING THE 5'UTR OF SURVIVAL MOTOR NEURON 2 (SMN2) TO INCREASE ITS EXPRESSION IN A DISEASE MODEL OF SPINAL MUSCULAR ATROPHY

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SMN2 is capable of producing full-length SMN protein, but does so at a relatively low rate due to exon 7 skipping in a majority of transcripts. Increasing the total number of SMN2 transcripts has the potential to compensate for loss of SMN1. The 5' and 3' untranslated regions (UTRs) of a gene contain cis-regulatory elements that modulate transcript stability and/or translational efficiency. We sought to determine whether the 5'UTR of SMN2 contains a repressive feature that limits its expression, targeting of which could increase SMN levels. Splice-switching oligonucleotides, such as nusinersen, have been successfully used to increase full-length SMN levels, but their effects are limited by the abundance of SMN2 transcripts in a cell. Thus, we further asked if targeting the 5'UTR and exon 7 splicing simultaneously could enhance SMN expression more than targeting either region alone. We identified an antisense oligonucleotide (ASO) complementary to the 5'UTR of SMN2 that increases SMN protein levels in SMA patient fibroblasts. Further, this ASO significantly increases SMN2 mRNA levels, but not pre-mRNA levels, suggesting the ASO offers protection to mature transcripts. Interestingly, 5'UTR ASO treatment shifts the SMN2 isoform ratio toward exon 7 inclusion, possibly through a feedback loop involving the SMN protein itself, which has a well-characterized role in spliceosome biogenesis. Combining the 5'UTR ASO with a previously developed splice-switching oligonucleotide results in a further increase in SMN protein levels. Future experiments will examine the mechanism of action of this ASO. Our results add to our current understanding of SMN regulation and may reveal a new therapeutic target for SMA.

NOTES

018P: DYSREGULATED SIGNALING IN SMA: FROM ISOLATED PATHWAY APPROACHES TO A CLUSTERED NETWORK REPRESENTATION

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Spinal Muscular Atrophy is caused by low levels of functional SMN protein. However, downstream disease mechanisms remain elusive. In the recent years several studies suggested a number of signaling pathways which mediate pathological changes in SMA. We identified molecular mechanisms linking SMN with altered profilin/ROCK signaling as well as with an enhanced ERK-activity. Moreover, we showed a connection between both pathways since ROCK inhibits ERK and vice versa. Co-inhibition experiments in SMA-mice demonstrated that this lateral connection between both signaling axes is relevant for the SMA-like pathophysiology. This indicates that an isolated pathway approach may be a rather reductionistic model of dysregulated signaling in SMA. Here, we employed a screening against phospho-proteins in pre-symptomatic and symptomatic SMA-mice which allows us to identify several dysregulated targets simultaneously. A bioinformatic analysis identified three clustered networks, growth factor signaling, MAPK signaling, and cell cycle / DNA repair which are interconnected. Identification of key-signaling nodes within each of these clusters is an important milestone which allows a rescue of SMA-like phenotypes alone or in combined drug approaches.

Acknowledgements
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NOTES

O19P: IMPROVED IN VITRO MODELS OF THE HUMAN BLOOD-BRAIN BARRIER (BBB) USING ENDOTHELIAL CELLS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS (IPSCS) FOR TESTING CNS THERAPEUTICS

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The blood-brain barrier (BBB) is primarily composed of highly specialised brain microvascular endothelial cells (BMECs), pericytes and end processes of astrocytes. The BBB tightly controls the exchange of molecules and cells between the brain and the blood. Although the BBB successfully maintains the brain microenvironment, it also blocks beneficial therapeutics for diseases of the central nervous system (CNS). The tight junction between the BMECs is the recognised phenotype of the BBB and is empirically defined by high trans-endothelial electrical resistance (TEER) and low permeability to paracellular markers. Models derived from animal tissue have proven useful, but suffer from relatively low TEER values and high permeability. We have produced improved in vitro models of the BBB using BMECs differentiated from human induced pluripotent stem cells (iPSCs). Three different clones of wild-type (4603, 19-9-7T and AD3-CL1) and a single clone of Spinal Muscular Atrophy type I (SMA I) iPSCs were differentiated into BMECs, characterised and assessed for proficiency to form BBB models. The integrity of the models was evaluated using TEER, expression of tight junction protein occludin, and permeability to paracellular markers lucifer yellow (LY) and sodium fluorescein (NaF). For comparison, the TEER of the most widely used in vitro model of the human BBB, made of the primary human brain endothelial cell line hCMEC/D3 (D3), was used.

The TEER values of 4603 and 19-9-7T derived BMECs are comparable to the value reported for co-culture models using endothelial cells, pericytes and astrocytes and over 60-fold higher than D3 cells. The SMA I iPSC-derived BMECs have higher TEER than AD3-CL1 iPSC-derived BMECs and 40-fold higher than D3 cells. The permeability of iPSC-derived BBB models to LY and NaF was 40-fold and 3-fold less than in the D3 model, respectively. Seven-fold more 4603 BMECs express glucose transporter-1 (GLUT-1) compared to D3 cells. All iPSC-derived BMECs express tight-junction protein occludin, whereas D3 cells do not. Our in vitro models of the BBB with BMECs alone display tight junction that closely mimics the human BBB in vivo and will have many potential uses including testing of therapeutic agents aimed at the CNS and investigating BBB breakdown in disease states. We are currently testing the crossing of therapeutics for SMA through our BBB models.

This work was funded by The SMA Trust through the UK SMA Research Consortium.

NOTES

SESSION 6 - EMERGING PHENOTYPES & STANDARDS OF CARE

020P: MRI OF THE CERVICAL SPINAL CORD AND NERVE ROOTS IN SMA

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OBJECTIVE:
What we know of SMA pathology is mainly based on post-mortem and animal studies. The early detection of changes in pathological mechanisms in SMA patients in vivo is increasingly important in light of therapeutic developments. Innovative MRI techniques could help to dissect pathological mechanisms and provide unique anatomical and functional biomarkers of disease progression or response to treatment. Therefore we assessed the feasibility of novel MRI protocols in SMA patients and healthy controls.

METHODS:
We developed 4 MRI protocols of the cervical spinal cord and nerve roots of spinal segments C3-C8 on a 3 Tesla MRI system. Two anatomical MRI protocols to investigate 1) cross-sectional area (CSA) at each spinal segment of the whole spinal cord and grey and white matter separately (Fig B) and 2) the diameter of anterior and posterior nerves at each spinal segment (Fig A) and two functional diffusion tensor imaging (DTI) protocols to assess the diffusion parameters fractional anisotropy (FA), mean (MD), axial (AD) and radial diffusivity (RD) of 1) segments C5-C7 of the cervical spinal cord (Fig C) and 2) cervical nerve roots (C3-C8) (Fig D). For all 4 protocols we investigated the difference in measures between patients and healthy controls.

RESULTS:
We included 10 patients with SMA types 2-3 and 20 age-and gender-matched healthy controls. We found an overall smaller CSA in patients compared to controls ($p=0.017$), with atrophy rates up to 8.5% at spinal segment C7. This difference seems mainly caused by a decrease in grey matter CSA. DTI data showed a slightly higher FA in the grey matter of patients compared to controls ($p=0.032$). Mean thickness of anterior nerves overall (C4-C8) was 0.93±0.13 mm in patients and 1.05±0.27 mm in controls. Thickness of posterior nerves was 1.03±0.22 in patients and 1.12±0.35 mm in controls. In the nerve roots (C3-C8) all DTI parameters were lower in patients compared to controls, but significant differences in MD, AD and RD were located at the rostral segments C3-C5 ($p<0.037$).

CONCLUSION:
We found differences between patients and controls using both structural and functional MRI protocols, confirming the potential of this technique to assess pathological mechanisms in SMA. When further developed and evaluated longitudinally in a larger group, it could provide novel biomarkers that can be implemented in therapy development and evaluation.

NOTES

021P: END OF STUDY RESULTS FROM ENDEAR: PROPORTIONS OF HINE-2 AND CHOP INTEND RESPONDERS

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BACKGROUND:
ENDEAR (NCT02193074) was a phase 3, randomized, double-blind, sham-procedure controlled 13-month study of the efficacy and safety of nusinersen in infantile-onset spinal muscular atrophy (SMA).

OBJECTIVE:
To report the likelihood of motor function responses among nusinersen-treated and control infants who were alive and remained in the ENDEAR study.

METHODS:
Symptomatic infants diagnosed with SMA (most likely to develop SMA Type I) were randomized (2:1) to receive intrathecal nusinersen (12-mg scaled equivalent dose) or sham-procedure. Eligibility criteria included: genetic diagnosis of 5q SMA, 2 SMN2 copies, age ≤6 months at symptom onset, and age ≤7 months with no hypoxemia at screening. The first primary endpoint was the proportion of Hammersmith Infant Neurological Examination section 2 (HINE-2) motor milestone responders excluding voluntary grasp, defined as those with (1) ≥2-point increase or maximal score in kicking ability or ≥1-point increase in head control, rolling, sitting, crawling, standing, or walking and (2) more HINE categories improving than worsening. A secondary endpoint was the proportion of Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) responders, defined as those with a ≥4-point improvement from baseline in CHOP INTEND score. Infants who died or withdrew from the study were not included in the current analyses.

RESULTS:
Of nusinersen- and sham procedure-treated infants alive at each time point, the likelihood of HINE-2 response was 51% (30/59) versus 9% (2/23) at Day 183, 61% (22/36) versus 0% (0/16) at Day 302, and 77% (20/26) versus 0% (0/11) at Day 394. When the later of Day 183, 302, or 394 assessments were used for each infant with a ≥Day 183 assessment, the overall likelihood of HINE-2 response was 64% (37/58) in nusinersen-treated infants versus 0% (0/20) in control infants. The likelihood of CHOP INTEND response followed a similar pattern with rates increasing over time in the nusinersen treatment group. Among nusinersen- and sham procedure-treated infants who were alive at each time point, 90% (52/58) versus 5% (1/20) were CHOP INTEND responders when the later of Day 183, 302, or 394 assessments was analyzed.

CONCLUSION:
The likelihood of response on motor function assessments increased over time on study. Most nusinersen-treated infants who were alive and remained in the study were responders on the HINE-2 (64%) and CHOP INTEND (90%) at their last study visit.

NOTES

022P: COGNITIVE DEVELOPMENT, LANGUAGE AND USE OF AUGMENTATIVE ALTERNATIVE COMMUNICATION IN SMA1 CHILDREN IN ITALY

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Today "technology dependent" SMA1 patients, with either invasive or non-invasive respiratory support, may reach their teens. It is known that extreme muscular weakness prevents SMA1 children from speaking clearly. Despite their vocalizations and glances, children are not always able to express what they think and this may make them angry and/or frustrated, even if there are many differences between one child to another. This is a very important issue because it affects the wholeness of the person. Nevertheless, the improvement of Communication is still not universally considered within Standards of Care.

The AAC PEPE-which is short for Augmentative Alternative Communication Program for Early Parental Empowerment, was developed for SMA1 patients in order to guarantee a model of AAC intervention targeted at the characteristics of SMA1. Early exposure to AAC as an input is in fact especially important for SMA1 children to experiment possible functions and to support internal language and experiences.

We decided to test fifteen children with SMA1 aged 3.8–11.2 years. One-dimensional Raven test (CPM) was used to evaluate cognitive development, and Brown Bellugy modified for Italian standards (TCGB) was used to evaluate language comprehension.

All 15 children collaborated to CPM, with an average IQ of 116. Eight children collaborated to TCGB, that was in the normal range in all of them. Children with an early AAC intervention scored in the higher range in both tests.
In 2009: 3 SMA1 children were involved in AAC PEPE
In 2017: 55 SMA1 children, 25 with early onset between 6 and 9 months, are involved in the project.
Young children are immediately exposed to listening to books with full text in symbols (Inbook), and by 3 years of age they are able to use advanced technological tools, can communicate independently and their communication is effective and rewarding.
We would like to point out how early investment in AAC influences abilities in: cognitive, linguistic, interaction, relationship, thought, active participation in daily life, decision-making, learning.

Thanks to the AAC project, the quality of life is improved of both SMA1 children and their families resulting in the acquisition of:

- higher IQ and better language comprehension
- extended interpersonal, communication skills
- stronger identity, greater independence
- increased self-esteem, self-efficiency
- development of cognitive potential in mainstream classes
- can drive power wheelchair with switches.

NOTES

023P: CLINICAL CHALLENGES IN THE TREATMENT OF SPINAL MUSCULAR ATROPHY (SMA) WITH NUSINERSEN

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In June 2017, the European Medicines Agency approved the antisense oligonucleotide (ASO) nusinersen for the treatment of all for all 5q-associated spinal muscular atrophy (SMA) types. The 2´-O-methoxyethyl phosphorothioate modified antisense drug nusinersen has to be administered intrathecally within an initial saturation period (day 0, 14, 28 and 63) to warrant drug saturation followed by a frequent maintaining application in 4 month intervals. Due to the clinical features of SMA, the clinical application of the ASO by lumbar punction is clinically challenging considering the frequently observed scoliosis, previous orthopedic surgeries or the need of sedation especially in infants or young children.

We are currently treating 19 patients with 5q-associated SMA (type 1 to 3) in the age ranging from 8 months up to 52 years, 7 with permanent or intermittent ventilatory support. We report from clinical observations and challenges regarding the treatment procedures that often require interdisciplinary collaborations (neurologists, neuroradiologists, pediatrics, orthopedics and anesthesiologists). Here, CT-graphic supported lumbar punction is the most challenging issue regarding drug application and was necessary in 8 of the patients. In detail, 4 of these 8 patients had prior lumbar spine surgery (e.g. spondylodesis) whereas a severe scoliosis was clinically evident in the other 4 patients that lead to frustrating lumbar punction without imaging support. Further, in infants and children, topical anesthetics and opioids (e.g. nalbuphine) in moderate dosage had to be administered to warrant successful lumbar punction. Indeed, adolescents and adults received solely topical and local anesthetics for lumbar punction with the exception of long-lasting punction procedures where patients had to be treated with benzodiazepines such as midazolame, opioids or anesthetics such as s-ketamine.

However, adolescent or adult SMA-patients with less severe disease progression are currently also under treatment with nusinersen in our department but the available therapy monitoring strategies and outcome variables regarding clinical efficacy such as motor scales seem not be appropriate considering that main motor functions have been clinically developed. Thus, we suggest further clinical and laboratory features to monitor the clinical efficacy especially in older and those with less disease progression such as cerebral spinal fluid, imaging and electrophysiological parameters.

NOTES

SESSION 7 - CHALLENGES OF CLINICAL TRIALS & BEYOND

024P: BENEFITS OF EARLIER TREATMENT WITH NUSINERSEN IN INFANTS AND CHILDREN WITH SPINAL MUSCULAR ATROPHY (SMA)

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BACKGROUND: Nusinersen has shown significant and clinically meaningful efficacy on motor milestone achievement and motor function in multiple SMA populations, on survival in infantile-onset SMA and a favourable safety profile.

OBJECTIVE: To evaluate the benefits of nusinersen based on age and disease duration at the time of screening in symptomatic children and infants who started treatment in a pre-symptomatic stage.

DESIGN/METHODS: ENDEAR and CHERISH were randomised, double-blind, sham procedure-controlled studies of nusinersen in infantile-onset SMA (most likely to develop SMA Type I) and later-onset SMA (most likely to develop SMA Type II or III), respectively. NURTURE is an ongoing, single-arm, open-label study of nusinersen in infants in a pre-symptomatic stage of SMA (most likely to develop SMA Type I or II). All study participants had documented 5q SMA. ENDEAR enrolled symptomatic infants age ≤7 months with onset of SMA at age ≤6 months and 2 SMN2 gene copies. CHERISH enrolled symptomatic children age 2–12 years with onset of SMA at age >6 months who had sat but never walked independently. NURTURE enrolled infants age ≤6 weeks and no clinical signs of SMA at first dose with 2 or 3 SMN2 gene copies.

RESULTS: In ENDEAR, higher proportions of HINE motor milestone responders were observed with nusinersen compared with sham control in the overall population and in subgroup analyses based on disease duration at screening and age at SMA onset (≤12, >12 weeks; Fig). In CHERISH, HFMSE LSM scores were significantly improved over 15 months with nusinersen versus control (Fig). Greater benefits were observed in younger children and those treated sooner after symptom onset (Fig). Similar results were noted for other ENDEAR and CHERISH endpoints. Mean HINE total motor milestone scores increased over time in nusinersen-treated infants in NURTURE. Infants with 2 SMN2 gene copies treated in the pre-symptomatic stage in NURTURE had higher mean HINE total motor milestone scores after ~1 year of treatment (mean[SE] score at Day 365: 19.7[1.7]) compared with ENDEAR infants who initiated treatment after symptom onset (mean[SE] score at Day 394: 7.19[1.0]).

CONCLUSIONS: Nusinersen showed consistent benefits on measures of motor function in infantile-onset SMA, later-onset SMA and in infants in a pre-symptomatic stage. Subgroup analyses indicate that initiation of nusinersen in infants/children with SMA earlier in their disease course maximises the treatment benefit.

NOTES

025P: CLINICAL EFFECTS OF NUSINERSEN INJECTIONS IN SMA TYPE 1 PATIENTS OLDER THAN 7 MONTHS: 10 MONTHS OF FOLLOW UP

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OBJECTIVE:
To evaluate safety, tolerability and clinical efficacy of nusinersen treatment in SMA type 1 patients older than 7 months.

INTRODUCTION:
Nusinersen is the first market approved treatment for spinal muscular atrophy. Clinical trial in SMA type 1 involved only patients younger than 7 months, therefore data are lacking in older patients, in which safety and efficacy have not been studied so far.
Methods: Since December 2016, we have started the treatment by nusinersen in 35 SMA type 1 patients older than 7 months (16 females and 19 males). Patients have been evaluated at pre-treatment, at two months of treatment and every 4 months subsequently.

We performed neurological examination and HINE (Hammersmith Infant Neurological Examination) motor milestones score. We noted weight gain, ventilatory and nutritional support, hospitalizations and side effects of the treatment. According to their age, patients were assessed by an experienced physiotherapist in a scale with CHOP Intend (Children's Hospital of Philadelphia), MFM (Motor Function Measure) 20 or 32, HFMS (Hammersmith Functional Motor Scale). In few cases, formal evaluation was not performed due to limited mobility or poor cooperation of patients.

RESULTS:
The median age at first injection was 24.5 months [8.3 – 113]. Median follow-up was 10.4 months [7.0 – 12.85]. 14 patients had 2 copies of SMN2, 19 patients had 3 copies and in 2 patients the number of copies was not quantified. Up to date, nusinersen was safe and well tolerated in our cohort. Patients presented with variable but significant and constant motor improvement. In 5 cases, sitting position was acquired. 12 patients improved by more than 2 points their HINE score. Patients' and family's quality of life was improved according to parents' declarations.

CONCLUSIONS:
Results in this cohort of older patients are in line with data obtained in younger patients during the phase 3 study (ENDEAR). More longitudinal data are mandatory to determine long-term benefit and cost-effectiveness of the treatment.

NOTES

026: MORE THAN JUST FUN AND GAMES: ACTIVE WORKSPACE VOLUME VIDEO GAME QUANTIFIES UPPER EXTREMITY FUNCTION IN INDIVIDUALS WITH SPINAL MUSCULAR ATROPHY (SMA)

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OBJECTIVE:
Investigate the validity and clinical meaningfulness of measuring upper extremity workspace volume and trunk control with a custom designed video game called ACTIVE.

BACKGROUND:
Accurate assessment and tracking of upper extremity function is critical in research and for long-term monitoring of functional abilities in the era of commercially-available treatments. ACTIVE can quantify reaching abilities across a range of patients from those limited to small hand movements to those independent in self-care activities.
ACTIVE, a 65-second video game, quantifies workspace volume (WSV) and trunk control on a continuous scale using a skeletal tracking algorithm. ACTIVE quantifies the volume of space that the player reaches (m3) and how far he/she leans (cm) as the player reaches over-head, forward and laterally to either squish spiders or dig for jewels. Scaled scores based on the player's wingspan can be compared to other patients or to accommodate for growth.

METHODS:
61 individuals with SMA and 385 age matched controls completed two ACTIVE trials, and a battery of traditional outcome measures appropriate for their age and diagnosis. These included the PROMIS parent report of upper extremity function, Six Minute Walk Test (6MWT), Hammersmith Functional Motor Scale Expanded (HFMSE), Revised Upper Limb Module (RULM).

RESULTS:
ACTIVE differentiated between patients with varying severity of SMA, as defined by their Brooke level, and healthy controls (Jonckheere-Terpstra test for trend $p<0.0001$). The functional relevance of ACTIVE WSV scores was demonstrated by highly significantly correlations with both the self-report (N=24) and parent proxy (N=33) version of the PROMIS (Rho=0.89, 0.75; $p<0.001$) Content validity was demonstrated by the high correlations between ACTIVE and the RULM (N=24; Rho=0.92; $p>0.01$) and HFMSE (N=39; Rho=0.91; $p>0.01$). To explore the concept of "minimal WSV" required to complete self-care activities we will present graphs from 5 PROMIS items comparing those who answered "Able with no trouble" and "Not able to do" to determine a minimum amount of volume necessary to complete these items. Longitudinal data will also be presented.

CONCLUSIONS:
ACTIVE can accurately quantify arm abilities and track meaningful functional changes in patients with spinal muscular atrophy. Its ease of use and objective quantification of abilities makes it an ideal choice for both research and clinical assessments.

NOTES

027P: FIREFISH, A MULTI-CENTER, OPEN-LABEL TRIAL TO INVESTIGATE THE SAFETY AND EFFICACY OF Rg7916 IN BABIES WITH TYPE 1 SMA: STUDY UPDATE AND REAL-LIFE EXPERIENCE OF STUDY IMPLEMENTATION

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SMA is caused by mutation or deletion of the survival of motor neuron 1 (SMN1) gene; a second SMN gene, SMN2, produces low levels of functional SMN protein. RG7916 is an oral, small-molecule SMN2 pre-mRNA splicing modifier that distributes into CNS and peripheral tissues and increases SMN protein levels.

FIREFISH (NCT02913482) is a multi-center, open-label, seamless pivotal study evaluating the safety and efficacy of RG7916 in babies aged 1–7 months at enrollment with Type 1 SMA and two SMN2 gene copies. The exploratory Part 1 (n=8–24) is assessing the safety, tolerability, pharmacokinetics and pharmacodynamics of RG7916 at different dose levels. In Part 1, patients receive RG7916 for at least 4 weeks (or 2 weeks after steady-state is achieved) of daily administration; patients then enter an extension phase with RG7916. The confirmatory Part 2 (n=40) will assess the safety and efficacy of RG7916 at the dose level selected from Part 1 over 24 months. The primary endpoint for Part 2 is the proportion of infants sitting without support for 5 seconds, assessed by the Gross Motor Scale of the BSID-III, after 12 months of treatment. As planned in the study protocol, a Safety Monitoring Committee regularly reviews all safety information from all FIREFISH participants.

At the time of abstract submission, no drug-related adverse events leading to study discontinuation have been observed in any patients with SMA receiving RG7916. In addition to a FIREFISH study update, real-life experiences and insights into conducting such a study in Type 1 SMA will be presented. These include coordinating a multi-disciplinary team of healthcare specialists dealing with such young babies, relocating families away from their home country and the importance of assuring standard-of-care practices whilst patients participate in the trial.

The FIREFISH study is ongoing and currently open for recruitment globally.

FIREFISH is sponsored by F. Hoffmann-La Roche.

NOTES

028P: A LONG-TERM, OPEN-LABEL FOLLOW-UP STUDY OF OLESOXIME IN PATIENTS WITH TYPE 2 OR NON-AMBULATORY TYPE 3 SPINAL MUSCULAR ATROPHY WHO PARTICIPATED IN A PLACEBO-CONTROLLED PHASE 2 TRIAL

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Olesoxime is an oral, daily administered compound that supports the function of mitochondria. In a previous randomized, double-blind Phase 2 study (NCT01302600) in patients aged 3–25 years with Type 2 or non-ambulatory Type 3 SMA, olesoxime maintained motor function over 24 months, whilst the placebo group declined. OLEOS (NCT02628743) is an open-label extension study (OLEOS; NCT02628743) assessing the long-term safety and efficacy of olesoxime in patients with Type 2 or non-ambulatory Type 3 spinal muscular atrophy (SMA).

One hundred and twenty-nine patients with Type 2 or non-ambulatory Type 3 SMA from previous Phase 2 study were enrolled and treated with olesoxime (10 mg/kg); the majority have been followed for 12 months (n=104). Primary endpoint is safety and secondary endpoints include change in Motor Function Measure (MFM) D1+D2 from baseline up to 5 years. OLEOS baseline visit occurred 2.4–5.1 years (median 3 years) after study drug discontinuation in Phase 2.
Consistent with previous studies, olesoxime was generally safe and well tolerated at the dose assessed. Maintenance of motor function observed over 2 years in the Phase 2 study was followed by a substantial decline in MFM D1+D2 (>2 points/year) after drug discontinuation. However, the ~2-point MFM treatment difference between olesoxime and placebo at the end of Phase 2 was maintained at OLEOS baseline. Furthermore, olesoxime open-label treatment stabilized motor function (mean change in MFM D1 + D2 from baseline: 6 months, -0.03 [SD, 4.79; n=124]; 12 months, -0.22 [SD, 4.74, n=104]). These data support the long-term stabilization of motor function observed in the Phase 2 study. A study update will be presented.

These data suggest that olesoxime offers the potential to provide meaningful clinical benefit and may play a role in the future therapeutic management of SMA.
Study sponsored by F. Hoffmann-La Roche

NOTES

Poster abstracts

PLEASE DO NOT TAKE PHOTOGRAPHS OF THE SLIDES OR POSTERS DURING THE CONGRESS

SESSION 1 - BASIC FUNCTION OF SMN

O2P: SPLICING ANALYSIS IN A ZEBRAFISH MODEL FOR SPINAL MUSCULAR ATROPHY IDENTIFIES TRANSCRIPTS IMPORTANT FOR MOTOR NEURON AND SCHWANN CELL FUNCTION

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It remains debated how deficiencies in the ubiquitously expressed Survival Motor Neuron (SMN) gene result in the degeneration of motor neuron (MNs) in Spinal Muscular Atrophy (SMA). One hypothesis suggests that MNs are selectively vulnerable to deficient pre-mRNA splicing, and that defects in associated Schwann cells contribute to MN degeneration in a non-cell autonomous manner. To identify aberrantly spliced transcripts that are crucial for survival and activity of motor neurons, our lab performed transcriptome analyses in a zebrafish SMA model. RNAseq analysis of SMN deficient FAC-sorted motor neurons and Schwann cells revealed 284 and 767 significant alternative splicing events, respectively. Among these events, we identified aberrantly spliced transcripts for srsf6b, which encodes a tissue-specific splice factor enriched in the ventral spinal cord. We generated zebrafish srsf6b mutants and found that they display severe motor axon branching defects. This suggests that srsf6b is essential for MN function and possibly amplifies the consequences of a general SMN-induced splicing defect particularly in MNs. Our transcriptome analysis also identified neurexin2a (nrxn2a) as a novel downstream target of SMN. Reduced SMN levels increase exon skipping in nrxn2a in both zebrafish and mouse SMA models. Maternal-zygotic nrxn2a zebrafish mutants exhibit early motor axonal pathfinding and synaptogenesis defects, which later develop into muscular atrophy at adult stages. Zygotic nrxn2a mutants show normal axon pathfinding but interestingly exhibit increased anxiety behavior in adulthood. Finally, we also obtained evidence for a non-cell autonomous contribution to MN degeneration. Using live calcium imaging in the transparent zebrafish embryos, we found that a SMN deficiency causes reduced excitability of Schwann cells. Importantly, transgenic restoration of SMN specifically in Schwann cells alleviated motor axonal pathfinding defects. Our preliminary data suggest that a novel candidate gene, which is down-regulated in SMN-deficient Schwann cells, contributes to MN degeneration in the zebrafish SMA model. Together, our findings provide further support for the hypothesis that defects in pre-mRNA splicing and non-cell autonomous contributions of Schwann cells are critical in SMA pathology. This project is supported by the National Medical Research Council (NMRC; CBRG/0046/2013), Singapore.

NOTES

P1: FUS IS NOT REQUIRED FOR SNRNP BIOGENESIS AND SMN LOCALIZATION TO NUCLEAR BODIES

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ALS and SMA are fatal neurological disorders that involve the selective degeneration of spinal motor neurons. SMA is a monogenic disorder caused by ubiquitous deficiency in the SMN protein. ALS is predominantly a sporadic disorder, but several gene mutations have been linked to familial cases. Genetic and molecular studies increasingly suggest that ALS and SMA may share common underlying mechanisms of disease. Accordingly, mutations in the RNA binding protein FUS are associated with some of the most aggressive, juvenile-onset forms of ALS and recent in vitro studies have shown that FUS depletion or ALS-linked FUS mutations disrupt the normal localization of SMN to nuclear bodies. Furthermore, FUS has been shown to associate with SMN as well as spliceosomal snRNPs whose biogenesis is SMN-dependent. However, the normal requirement of FUS for snRNP biogenesis and the contribution of SMN dysfunction to FUS-ALS pathology remain unknown._

To investigate whether FUS and SMN are functionally linked, we studied the effects of FUS depletion on the localization and function of SMN using a comprehensive set of molecular, biochemical and immunohistochemical approaches in mouse models. Analysis of tissues from wild type and FUS knockout mice showed that SMN activity in snRNP assembly and the levels of endogenous spliceosomal snRNPs are not affected by FUS depletion. _Characterization of a panel of molecular signatures representative of diverse SMN-dependent RNA processing pathways that are dysregulated in SMA also revealed no differences between wild type and FUS knockout mice. Immunohistochemistry of mouse spinal cords further showed that FUS depletion did not affect SMN localization to nuclear Gems in motor neurons. Additionally, the nuclear levels of snRNPs and their enrichment in Cajal bodies were similar in motor neurons from wild type and FUS knockout mice.

Our results indicate that FUS is not required for the activity of SMN in snRNP assembly and that FUS ablation neither affects snRNP biogenesis nor SMN localization to nuclear bodies in motor neurons in vivo. Together with our previous work (Sharma et al 2016 Nat Commun), these findings argue against disruption of snRNP biogenesis through FUS loss-of-function as a potential mechanism for ALS pathogenesis. It remains to be established whether ALS-linked FUS mutations result in the gain of toxic properties that contribute to motor neuron disease by interfering with SMN functions.

NOTES

P2: CBP-MEDIATED SMN ACETYLATION MODULATES CAJAL BODY BIOGENESIS AND THE CYTOPLASMIC TARGETING OF SMN

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The survival of motor neuron (SMN) protein plays an essential role in the biogenesis of spliceosomal snRNPs and the molecular assembly of Cajal bodies (Cbs). Deletion of or mutations in the SMN1_ gene cause spinal muscular atrophy (SMA) with degeneration and loss of motor neurons. Reduced SMN levels in SMA lead to deficient snRNP biogenesis with consequent splicing pathology. Here, we demonstrate that SMN is a novel and specific target of the acetyltransferase CBP (CREB-binding protein). Furthermore, we identify lysine (K) 119 as the main acetylation site in SMN. Importantly, SMN acetylation enhances its cytoplasmic localization, causes depletion of CBs, and reduces the accumulation of snRNPs in nuclear speckles. In contrast, the__acetylation-deficient SMNK119R mutant promotes formation of CBs and a novel category of promyelocytic leukemia (PML) bodies enriched in this protein. Acetylation increases the half-life of SMN protein, reduces its cytoplasmic diffusion rate and modifies its interactome. Hence, SMN acetylation leads to its dysfunction, which explains the ineffectiveness of HDAC (histone deacetylases) inhibitors in SMA therapy despite their potential to increase SMN levels.

NOTES

P3: PHOSPHATASE AND TENSIN HOMOLOGUE (PTEN) DEPHOSPHORYLATES THE SPINAL MUSCULAR ATROPHY-DETERMINING GENE PRODUCT SMN

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Increasing SMN stability is of greatest therapeutic interest in SMA. The function and stability of SMN is partially regulated by phosphorylation. However, only few kinases and phosphatases acting on SMN are described. Additionally, the role of SMN's C-terminal phospho-sites is still elusive since this terminus is not easily accessible for proteinases used in mass spectrometry. Thus, characterising kinases and phosphatases which increase SMN stability would push forward the understanding of the SMA-determining gene product. Using microarray technology in a cellular SMA model, we showed that the PI3K/PTEN axis may be disturbed. Thus, we wondered whether PTEN is a phosphatase for SMN regulating its stability and function.

Here, we show by co-immunoprecipitation and co-localisation analyses that PTEN endogenously binds SMN as well as SMNΔ7 at the C-terminus within residues 235-278. Patient mutations (i.e. Y272C and T274I) localised within this sequence did not alter PTEN-SMN interaction. Interestingly, mimicking phosphorylation at the putative phospho-site S290 (S290D) disrupted PTEN binding whereas the non-phospho mutant (S290A) interacted with PTEN. To test whether PTEN not only binds but also dephosphorylates SMN, we employed a PTEN inhibitor together with 2D-gel electrophoresis confirming that PTEN is a novel phosphatase for SMN. Next, we wondered about the functional outcome of missing SMN dephosphorylation by PTEN. SMN protein but not transcript levels were decreased upon PTEN inhibition or knockdown in line with diminished numbers of SMN-positive nuclear bodies. To further elucidate whether decreased SMN stability is due to reduced dephosphorylation by PTEN or whether PTEN is involved in SMN translation, we performed pulse-chase experiments and could validate a post-translational function for PTEN on SMN. Testing whether PTEN depletion also destabilises SMN in vivo is part of ongoing studies.

Together, our data suggest that altered PI3K/PTEN signalling alters SMN phosphorylation, stability and function based on a direct molecular interaction of PTEN with SMN. Identification of the phospho-site(s) and the responsible kinase will open a novel strategy of modulating SMN stability and function.

This work was supported by the Niedersachsen-Research Network on Neuroinfectiology (N-RENNT) of the Ministry of Science and Culture of Lower Saxony, the Initiative SMA, and the Deutsche Muskelstiftung/Philipp & Freunde - SMA Deutschland

NOTES

P4: REPURPOSING OF DRUGS USING SMN2 DROSOPHILA SPLICEOSENSOR MODEL

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Spinal Muscular Atrophy is a rare and fatal neuromuscular disorder caused by the loss or reduction in the Survival Motor Neuron (SMN) protein levels. The affected individuals have mutated SMN1 gene and unaffected human specific SMN2 copy, which only partially is translated into a functional SMN protein. Pharmacological activation of SMN2 exon 7 inclusion by small molecules or modified antisense oligonucleotides (Spinraza™) is a promising approach to treat SMA. Here we describe a reporter system, informative of SMN2 exon 7 splicing modulation in Drosophila motor neurons. It was used for the screening of 1100 drugs from Prestwick Chemical Library, revealing numerous hit molecules. The most promising drugs were validated in SMA patient-derived fibroblasts where they proved to modify SMN2 splicing, thus becoming our candidates for further evaluation.

NOTES

P5: MODULATION OF THE PHOSPHORYLATION STATE OF THE SURVIVAL OF MOTONEURON PROTEIN SMN: IMPACT ON LOCALISATION AND STABILITY

Nora Tula Detering, Sebastian Rademacher, Inga-Maria Wefel, Peter Claus

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Posttranslational modification of proteins regulates important functional effects. The survival of motoneuron (SMN) protein directly binds to the phosphatase and tensin homologue (PTEN). PTEN acts as phosphatase for SMN (Rademacher et al., in preparation). Furthermore, pharmacological inhibition of PTEN resulted in decreased protein levels of SMN. In additional studies, PTEN knockdown by siRNA showed functional alterations of SMN, including inhibition of neurite outgrowth and reduced numbers of SMN-positive nuclear bodies. Therefore, altering the phosphorylation state of specific sites of SMN by PTEN might impact the stability of SMN. However, little is known about the overall phosphorylation state of SMN and its potential role in SMN localisation and stability. Accordingly, determining the relevant phosphorylation sites of SMN is indispensable to understand function and stability of SMN.

In this study, we investigated the impact of phosphorylation on localisation and stability of SMN by generating SMN phospho-mutants. We have generated a library of phospho-mimicking and non-phosphoryable SMN mutants based on known phosphorylation sites, our own mass spectrometry analyses and computational predictions. The localisation of SMN mutants in nuclear bodies was quantified as it is one indicator for the stability of SMN. In addition, we analysed the effect of PTEN: Given that the phosphatase PTEN increases the stability of SMN by dephosphorylation, altered nuclear localisation of SMN would subsequently be expected under PTEN knockdown. _By this approach, we identified distinct phospho-mutants which showed a rescued number of SMN-positive nuclear bodies under PTEN knockdown. Hence, these phosphorylation sites of SMN can be potentially relevant for regulation of localisation and stability of SMN.___

Acknowledgements: This work was supported by the Niedersachsen-Research Network on Neuroinfectiology (N-RENNT) of the Ministry of Science and Culture of Lower Saxony, the Initiative SMA, and the Deutsche Muskelstiftung/Philipp & Freunde - SMA Deutschland.

NOTES

P6: SMN DOES NOT LOCALISE TO STRESS GRANULES BUT REGULATES THEIR FORMATION

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Stress granules (SGs) are cytoplasmic complexes comprising mRNA molecules and different proteins. They are formed as a reaction to cellular stress. RNA molecules not essential for cellular survival are stored in SGs thereby preventing their translation. This is an energy-saving process and important for regeneration and survival after removal of the stressor. Thus, SGs are dynamic structures that assemble under stress and dissolve after stress elimination. However, SGs often become permanent aggregates in neurodegenerative diseases.

Previous studies in fibroblasts suggested that SMN localises to SGs. However, combining confocal microscopy with in-depth image analyses, we showed that SMN does not localise to stress granules in motoneuron-like NSC34 cells. Interestingly, we demonstrated a co-localization of SMN-interacting proteins Profilin 1 and Profilin 2, with SGs. Therefore, we hypothesised that SG dynamics are indirectly altered in SMA. SMN reduction changes steady state levels of Profilins eventually resulting in a functional loss of SGs. To investigate this, we used a high-throughput technology for SG quantification in NSC34 cells. Interestingly, a siRNA mediated knock-down of SMN resulted in a significant increase of SG numbers in stressed NSC34 cells. In conclusion, SMN regulates SG dynamics in motoneuron-like cells.

Acknowledgements: This work was supported by the Niedersachsen-Research Network on Neuroinfectiology (N-RENNT) of the Ministry of Science and Culture of Lower Saxony, the Initiative SMA, and the Deutsche Muskelstiftung/Philipp & Freunde - SMA Deutschland, and SMA Europe.

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P7: SMN AND TRANSLATION AT SINGLE NUCLEOTIDE RESOLUTION

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Spinal Muscular Atrophy (SMA) is a progressive neurological disorder caused by low levels of SMN protein. We recently demonstrated that SMN loss causes defects in ribosome biology in vivo, suggesting that SMN can play a direct role within the translational machinery. Therefore, SMN loss can be mechanistically connected to pathological dysregulation of protein synthesis in SMA.

To investigate the direct role of SMN in translation and any dysregulation associated with its loss that leads to SMA, we studied the possible mislocalization of ribosomes caused by reduced levels of SMN using ribosome profiling. This technique is based on the identification of RNA fragments protected by ribosomes from endonuclease digestion, allowing to investigate ribosome positions at single nucleotide resolution at a genome-wide scale. We performed ribosome profiling in brains from early-symptomatic and littermate control mice collected at post-natal day 5.

By developing an experimental assay to sequence ribosome protected fragments isolated from SMN-associated ribosomes and a dedicated computational pipeline for the extraction of positional information, we observed that SMN-interacting ribosomes protect transcripts associated with SMA. Moreover, in early-symptomatic tissues we revealed positional defects in ribosomes along the coding sequence, caused by the loss of SMN.

Overall, these findings suggest a critical role of SMN in regulating polysome activity at multiple levels.

NOTES

SESSION 2 - NEURONAL-SPECIFIC FUNCTION OF SMN

O4P: DEFINING CONSERVED GENE NETWORKS AFFECTED IN SPINAL MUSCULAR ATROPHY USING DROSOPHILA MODEL

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Spinal Muscular Atrophy (SMA) is a devastating inherited disorder characterized by progressive loss of motor activity due to death of motor neurons, structural and functional failure of neuromuscular synapses and muscle atrophy. The cause of SMA is primarily reduction of Survival Motor Neuron (SMN) protein in spinal cord motor neuron due to mutation of SMN1 gene, so restoring SMN protein levels in spinal cord motor neuron has been primary target of therapeutic approaches. However, underlying mechanism that manifest SMA pathological phenotypes are not well understood. Furthermore, gene networks that link low levels of SMN expression and SMA pathologies is remained poorly understood. We have sought to address this extant question by using Drosophila SMA model. Elimination of Smn in Drosophila ovary by germline recombination resulted in developmental arrest at the early stages of oogenesis. Homozygous Smn deletion mutant survives through embryo genesis but dies during early larval stages as diminishing maternal Smn. Zygotic knockdown of Smn using RNA interference exhibited phenotypes similar to pathological phenotypes seen in human SMA patients: such as loss of motor axons, degeneration of muscles and defects in the neuromuscular junction. Moreover, the severity of mutant phenotype depends on Smn protein dose. Our genetic screens revealed over 300 genes may functionally interact with Smn. Further analysis focused on two canonical pathways, BMP and FGF signaling pathways, indicated that modulation of trans-synaptic signaling can rescue the Smn defects. As we found that FGF and BMP pathways modulate distinct aspects of SMA motor neuron and NMJ pathologies, we examined if reduction SMN levels alter expression of any known components of those pathways by quantitative PCR assay targeting isoforms of 16 components of those pathways and found that expression of only one or two transcriptional isoforms of a few genes were altered. As SMN protein is a core component of the Gemin complex and support biogenesis of RNA processing machinery, we have been examining the impact of Smn mutations on global gene expression and splicing patterns in CNS of Drosophila SMN mutant by transcriptome analysis. In addition, we are analyzing and comparing those data with RNAseq data that we obtained from SMA patient fibrobrast driven motor neuron cells to defining conserved pathway between human SMA and Drosophila SMA model.

NOTES

05P: NEURONAL ACTIVITY REGULATES DROSHA VIA AUTOPHAGY IN SPINAL MUSCULAR ATROPHY

Inês do Carmo Gonçalves[1], Johanna Brecht[1], Maximilian Thelen[1], Wiebke Rehorst[1], Miriam Peters[1], Laura Torres-Benito[1], Darius Ebrahimi-Fakhari[2], Natalia Kononenko[3], Mustafa Sahin[2], Brunhilde Wirth[1], **Min Jeong Kye[1]**

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Dysregulated miRNA expression and mutation of genes involved in miRNA biogenesis have been reported in motor neuron diseases including spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS). Therefore, identifying molecular mechanisms governing miRNA expression is important to understand these diseases. Here, we report that expression of DROSHA, which is a critical enzyme in the microprocessor complex and essential for miRNA biogenesis, is reduced in motor neurons from a SMA mouse model. DROSHA proteins are degraded by neuronal activity induced autophagy machinery, which is also abnormally regulated in SMA. Blocking neuronal activity or the autophagy-lysosome pathway restores DROSHA levels in SMA motor neurons. Moreover, reducing DROSHA levels enhances axonal growth. As impaired axonal growth is a well described phenotype of SMA motor neurons, these data suggest that DROSHA reduction by autophagy may mitigate the phenotype of SMA. In summary, these findings suggest that autophagy regulates RNA metabolism and neuronal growth via the DROSHA/miRNA pathway as a compensatory mechanism in SMA.

NOTES

O6P: TEMPORAL AND TISSUE VARIABILITY OF SMN PROTEIN LEVELS IN MOUSE MODELS OF SMA

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Spinal muscular atrophy is caused by deleterious variants in SMN1 that lead to a loss of full-length SMN protein expression. A second, almost identical SMN gene, called SMN2, is alternatively spliced, leading to truncated mRNA, the protein product of which is unstable and quickly degraded. A minority (~10%) of SMN2-derived mRNA, however, contains the full SMN sequence and is translated into stable, full-length SMN protein. Previous studies have shown that SMN expression requirements vary during development. Moreover, SMN expression is required in all tissues, but as SMA is primarily a motor neuron disease tissue requirements might vary. Surprisingly, little is known about the amount of full-length SMN protein that is derived from SMN2 mRNA and how protein expression varies over time and between tissues. In this study, we aimed to address this issue by determining absolute levels of SMN protein expression in 6 healthy control mice tissues (brain, spinal cord, muscle [m. gastrocnemius], heart, liver and kidney) at 3 developmental time points, matching pre-, early- and late-symptomatic stages of disease in mouse models of SMA. Next, we compared the relative amounts of SMN in healthy controls to that of affected littermates in 2 different mouse models of SMA ('Taiwanese' and SMN2B) in the same set of tissues. We found that, in control tissue, the absolute amount of SMN varies greatly over time and across tissues. Moreover, the level of SMN depletion in SMA models varies between the tissues investigated and between the 2 different models. These results suggest that SMN requirements likely correlate with the severity in which tissues are affected. Moreover, our results suggest that, despite a constant genetic background in all tissues, cellular mechanisms must be in place that lead to considerable variability in SMN protein levels based on developmental stage and tissue requirements. Studying the pathways that regulate the variability in SMN expression is likely to provide interesting new insights into SMA pathogenesis. Also, our findings have implications when designing and interpreting results from experimental as well as therapeutic studies.

NOTES

P8: METALLOPROTEASE-MEDIATED CLEAVAGE OF THE SEMAPHORIN RECEPTOR PLEXIND1 AND ITS SEQUESTRATION TO ACTIN-COFILIN RODS IN SPINAL MUSCULAR ATROPHY (SMA)

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Spinal muscular atrophy (SMA) is a neurodegenerative disease caused by the loss of the Survival of motoneuron-1 (SMN1) gene and is mainly characterised by degeneration of spinal motoneurons followed by muscle atrophy and weakness. We and others showed that dysregulation of the actin cytoskeleton, aberrant axonal guidance and altered maintenance of neuromuscular junctions contribute to muscle denervation which can be linked to hyperactive RhoA/ROCK signalling. However, upstream mechanisms leading to ROCK hyperactivation are still elusive. Here, we analysed the role of the guidance receptor PlexinD1 as a potential upstream effector since this molecule is important for axonal guidance, neuromuscular junction maintenance and control of monosynaptic specificity of reflex arcs.

We demonstrated that PlexinD1 is cleaved by metalloproteases in SMN-depleted motoneuron-like NSC34 cells. This PlexinD1 cleavage product binds to actin-cofilin (AC) rods, i.e. pathological actin and cofilin-containing structures known from other neurodegenerative diseases and congenital myopathies. PlexinD1-decorated AC rods were also detected pre-symptomatically in motoneurons of SMA mice from a severe as well as an intermediate SMA mouse model and in induced pluripotent stem cell (iPSC)-derived motoneurons from SMA patients. However, those structures were neither detected in skeletal or heart muscle from symptomatic SMA mice nor in SMN-depleted myoblast C2C12 cells indicating that the presence of PlexinD1-positive AC rods is motoneuron-specific. Furthermore, fragmentation of PlexinD1 results in altered cellular response to its specific ligand Semaphorin3E as assessed by growth cone collapse and could be corrected by the application of a metalloprotease inhibitor in vitro.

Together, our data suggest that AC rods contribute to SMA pathology and that inhibition of metalloprotease-mediated cleavage of PlexinD1 opens a novel strategy to correct aberrant cytoskeletal regulation, axonal guidance, motor endplate maintenance and malformation of reflex arcs.

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NOTES

P9: SMN COLOCALIZES WITH THE CYTOSKELETON IN MOTOR AXONS AND NERVE TERMINALS

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Spinal Muscular Atrophy (SMA) is a neurodegenerative disease characterized by the loss of spinal cord α-motoneurons, muscle weakness and progressive paralysis. SMN, the defective protein in the disease, participates in mRNA metabolism through snRNPs and mRNPs assembly. In addition, SMN is present in motor axons and participates in mRNP trafficking.

To get a deeper insight into the axonal and presynaptic role of SMN, we investigated the distribution and properties of axonal and presynaptic SMN particles in control and SMA mice. The study was performed in wild-type and two transgenic SMA mouse models, which express full length and truncated SMN proteins in different amounts. SMN expression was studied by quantitative confocal microscopy and western blot at different stages of synaptic maturation. We found that SMN granules colocalize with neurofilaments (NFs) and MAP1B in both motor axons and nerve terminals of control and mutant mice. In presynaptic terminals of SMA mice, SMN granules form large aggregates within the NFs accumulations.

In summary, these data show that SMN granules are associated with the cytoskeleton in motor nerve terminals and also demonstrate granules accumulation in SMA terminals, suggesting that SMN clearance is altered in the disease.

Financial support: Tatiana Perez de Guzman El Bueno Foundation and MINECO/FEDER Spanish grants (BFU2016-78934-P).

NOTES

P10: ROLE OF SMN IN THE AXONAL TRAFFICKING OF ANXA2 mRNA

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Spinal muscular atrophy (SMA) is mainly due to mutation and/or deletions of the survival motor neuron gene (SMN1). Besides its function in the biogenesis of spliceosomal snRNPs, there is compelling evidence for a role of SMN in axonal RNA trafficking and for its association with proteins involved in the regulation of mRNA transport, stability and/or local translation. Accordingly, SMN colocalizes with several axonal mRNAs of differentiated NSC-34 motor neuron-like cells (Rage et al., 2013). We recently showed that SMN depletion gives rise to a decrease in the axonal transport of the mRNAs encoding Annexin A2 (Anxa2). We also characterized the structural features of the Anxa2 mRNA required for its axonal targeting by SMN and found that a G-rich motif located near the 3'-UTR is responsible for its axonal transport by the SMN complex (Rihan et al., 2017). Our next goal is to visualize Anxa2 mRNA local translation in axons of the NSC34 motor neuron cell line. For this, we used the CRISPR-CAS9 strategy to insert a SunTag cassette into the Anxa2 gene. We are currently adapting the system to simultaneously visualize the Anxa2 mRNA and the nascent protein in living cells. Our work should help to better understand the role of SMN in the local translation of Anxa2 along axons of motor neurons.

NOTES

P11: DEPLETION OF PROFILIN2 BUT NOT PROFILIN1 PROMOTES COFLIN-ACTIN ROD FORMATION IN SPINAL MUSCULAR ATROPHY

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Although it has been known since the 90s that a lack of the SMN protein causes the specific SMA phenotype, the cause of the motor neuron susceptibility remains elusive. A link to the involvement of the actin cytoskeleton was made by demonstrating the direct interaction between SMN and the actin-binding protein profilin1/2 (PFN), of which PFN1 is also known as a risk gene for Amyotrophic Lateral Sclerosis, another motoneuron disease. Moreover, the RhoA/ROCK pathway, a key pathway in the regulation of the actin cytoskeleton, is hyperactive in SMA leading to aberrant phosphorylation of PFN2 and cofilin (Nölle et al., 2011, Hum. Mol. Genet.,Hensel and Claus, 2017, Neuroscientist) . Additionally, we have shown the presence of cofilin-actin (AC) rods in several cellular and mouse SMA models which are decorated with the intracellular cleavage fragment of the surface receptor PlexinD1 (Rademacher et al., 2017, Hum. Mol. Genet.). The formation of AC rods is promoted by oxidative stress and hyperactivation (i.e. hypophosphorylation) of cofilin, factors both found dysregulated in SMA.

Here, we analysed whether PFN1/2 together with cofilin promotes AC rod formation due to altered phosphorylation mediated by the hyperactivated RhoA/ROCK pathway. To test this, we applied the ROCK inhibitor Y27632 to control or SMN-depleted NSC34 cells and quantified the number of cells displaying AC rods. According to our hypothesis, ROCK inhibition reduced their number. To further investigate the role of ROCK downstream targets, we knocked down either PFN1 or PFN2. Interestingly, PFN1 knockdown had no influence on AC rod numbers whereas PFN2 knockdown led to a significant increase. Moreover, transfection of phosphomutants of PFN2 showed the contribution of specific phospho-sites of PFN2 to AC rod formation.

Together, our results indicate that ROCK inhibition not only ameliorates SMA phenotypes as previously shown but also reduces the number of AC rods. Cofilin hypophosphorylation drives the cell towards AC rod formation whereas PFN2 may be necessary for their removal. Phosphorylation of PFN2 at specific sites may inhibit this function.

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NOTES

P12: MODELLING SPINAL MUSCULAR ATROPHY (SMA) WITH INDUCED PLURIPOTENT STEM CELL DERIVED NEURONS

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BACKGROUND:
Spinal muscular atrophy (SMA) is an early onset motor neuron disease caused by deficiency of the SMN protein. Recent developments in stem cell technology have substantially expanded the range of cellular models available for motor neuron diseases by allowing the direct observation of pathological mechanisms in induced pluripotent stem cell (iPSCs)-derived neurons differentiated from fibroblasts from patients with the disease-specific mutation. The aim of this study is to develop a consistent and disease relevant phenotypic readout in iPSCs-MNs to facilitate screening for therapeutic targets and the evaluation of candidate molecules of potential therapeutic benefit.

RESULTS:
We generated iPSCs by reprogramming fibroblasts from Type II and Type III SMA patients. In both SMA iPSCs and iPSC derived MNs, SMN protein is decreased compared to cells from unaffected age-matched individuals. Decreased levels of SMN protein is correlated with high levels of cleaved caspase-3 and cytochrome c in SMA MNs, indicating that SMA MNs might be undergoing caspase-dependent apoptosis. _Furthermore, MNs from SMA patients also showed high levels of SQST1/p62, suggesting disrupted protein degradation under basal conditions compared to healthy controls.

CONCLUSIONS:
Our study identified cellular phenotypes that associate with decrease of SMN protein in SMA MNs and molecular changes that may account for their vulnerability in pathological conditions.

ACKNOWLEDGMENTS:
This work is supported by a research grant from The SMA Trust.

NOTES

P13: PLASTIC CHANGES IN C-BOUTON TYPE SYNAPTIC AFFERENTS OCCUR IN CONJUNCTION WITH GLIAL CELL RESPONSE IN SPINAL CORD MOTOR NEURONS AFTER INJURY

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Motor neuron (MN) excitability is modulated by cholinergic inputs through C-type afferent synaptic boutons, which display an endoplasmic reticulum-related subsurface cistern (SSC) adjacent to the postsynaptic membrane. Besides cholinergic molecules, a constellation of proteins involved in different signal-transduction pathways are clustered at C-type synaptic sites (M2 muscarinic receptors, Kv2.1 potassium channels, Ca2+ activated K+ [SK] channels, and sigma-1 receptors [S1R]). We have previously described that neuregulin-1 (NRG1)/ErbBs-based retrograde signaling occurs at this synapse. The relevance of NRG1/ErbB pathway is strengthened by the recent discovery of a new type of familial ALS caused by a loss of function mutation of ErbB4. C-boutons are lacking on the ALS-resistant oculomotor MNs. To better understand signaling through C-boutons, we performed an analysis of the distribution of C-bouton-associated signaling proteins in normal and altered MNs. We show that within SSC, S1R, Kv2.1 and NRG1 are clustered in highly specific, non-overlapping, microdomains, whereas ErbB2 and ErbB4 are present in the adjacent presynaptic compartment. This organization may define highly ordered and spatially restricted sites for different signal-transduction pathways in normal MNs. However, SSC associated proteins are seriously disrupted in axotomized MNs together with the activation of microglia, which display a positive chemotactism to C-bouton sites. The microglial activation and C-bouton disruption start 24h post-axotomy, reach its maximum at 2 weeks and progressively decline later, with a partial recovery after 30 days. This is found in conjunction with an astroglial reaction that shows an opposite time course profile respect to the microglia. Astrogliosis remains prominent 6 months after injury and even appears to be extended to neighbor areas of non-lesioned spinal cord. This indicates that C-bouton associated molecules are also involved in neuroinflammatory signaling in diseased MNs, emerging as new potential therapeutic targets.

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NOTES

P14: CELLULAR AND MOLECULAR ANATOMY OF THE HUMAN NEUROMUSCULAR JUNCTION: RELEVANCE TO SPINAL MUSCULAR ATROPHY (SMA) RESEARCH

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The neuromuscular junction (NMJ) is a chemical synapse responsible for signal transmission between a motor nerve terminal and its target skeletal muscle fibre, inducing muscle contraction. It is known to be a pathological target in numerous neurodegenerative conditions, including spinal muscular atrophy (SMA). Abnormalities in NMJs, including nerve terminal degeneration and immature motor endplates, have been observed in SMA mice and confirmed in SMA patients. However, most cellular and molecular studies of NMJ pathology in neuromuscular diseases such as SMA have relied heavily on 'model' organisms, both vertebrate and invertebrate, working on the tacit assumption that the biological principles uncovered can ultimately be applied to humans. Surprisingly, there is currently a relative paucity of data concerning the cellular and molecular composition of the human NMJ. Here, we combined morphological techniques, super-resolution imaging and proteomic profiling to compare the detailed cellular and molecular architecture of the healthy adult human NMJ with NMJs from rodent models. Human NMJs were significantly smaller, less complex and more fragmented than mouse NMJs. In contrast to mice, human NMJs were also remarkably stable across the entire adult lifespan, showing no signs of agerelated degeneration or remodelling. Super-resolution imaging (dSTORM) and proteomic profiling revealed a distinctive distribution of active zone proteins (SNAP25) and differential expression of core synaptic proteins and molecular pathways at the human NMJ. These findings reveal human-specific cellular and molecular features of the healthy NMJ that distinguish them from comparable synapses in other mammalian species. Such differences need to be taken into account when translating NMJ-based research findings from studies using animal models into human patients.

NOTES

P15: THE INTERMEDIATE FILAMENT PROTEIN, LAMIN A/C, IS DYSREGULATED IN SPINAL MUSCULAR ATROPHY

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Lamins are intermediate filament proteins of nuclear lamina that provide structural suport for the nucleus and regulate a variety of nuclear functions. Mutations in LMNA, the lamin A/C encoding gene, are a known cause of adult form of proximal SMA, and in a meta-analysis study of published proteomic data sets, we found that lamin A/C expression was consistently dyregulated in SMA across three separate studies. Here, we sought to biochemically verify the differential expression of lamin A/C in SMA, and to investigate the likely functional consequences of this in cultured SMA patient fibroblast cells. Quantitative western blot analysis confirmed that lamin A/C expression is dysregulated in SMA patient fibroblast cell lines and in a range of tissues from a severe mouse model of SMA. In addition, immunostaining of SMA patient fibroblasts revealed that SMN is sometimes present alongside of lamin A/C at the nuclear rim. _Overexpression or deep knockdown of lamin A/C was previously shown to affect cell proliferation, differentiation and migration in normal fibroblast cells. Contrary to this, we show that cellular proliferation and migration are not affected in Type 1 SMA patient fibroblast cells; suggesting that a greater change in lamin A/C expression is necessary to produce such functional defects. _As age-related changes in lamin A/C expression have been described in some cell types, current work in our laboratory aims to determine whether age exacerbates the perturbation of lamin A/C in SMA, leading to the emergence of functional impairments. In conclusion, widespread perturbations in lamin A/C expression, identified across a range of SMA tissues, implicate that lamin A/C might be an important regulator of disease pathogenesis. Therapeutic strategies for pharmacological or genetic manipulation of lamin A/C may offer the potential to complement strategies directed solely at SMN.

NOTES

SESSION 3 - SMA AS A SYSTEMIC DISEASE

08P: THE DEVELOPMENT OF HEART DEFECTS IN A MOUSE MODEL OF SEVERE SMA

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Heart defects are consistently described in patients and animal models of Spinal Muscular Atrophy (SMA), but we have no clear understanding of their aetiology. Here, we have carried out a systematic and detailed morphological assessment of heart pathology in the Taiwanese mouse model of severe SMA, focusing on events prior to the appearance of overt neuromuscular symptoms. Hearts are grossly normal and their weight relative to body weight is not significantly different between SMA and Het control mice from P1 (birth) to P8 (late symptomatic) timepoints. However, gross histological differences are apparent as ventricles are significantly enlarged with a thin interventricular septum at P3 and P5, while left ventricular wall is thinner at P1, P3 and P5 in SMA compared to control mice.

Immunohistochemistry revealed that ordering of the cardiomyocytes making up the heart walls is altered: first, the tri-laminar, spiral arrangement of cardiomyocytes essential for efficient ventricular contraction and emptying is not apparent in SMA, rather an embryonic circumferential arrangement is present. This apparent developmental delay is preceded by a decrease in expression of essential basement membrane protein collagen IV at P1; second, the embryonic trabecular structure of the ventricular luminal surface persists until P5 in SMA but not control hearts. Together, these defects describe a pattern of delayed development, as seen in other organs.

We found no differences in cell proliferation between P1 and P5, but significantly elevated cell death at P5, which was preceded by evidence of oxidative stress at P3 and P5, suggesting that degenerative changes my become important at later stages of development.

Significantly more blood is found pooled in SMA hearts in this fixed but non-perfused state, particularly in the ventricles between P1 and P5. This suggests that the defects reported in the heart wall are associated with decreased functionality, resulting in incomplete ventricular emptying. Taken together with previously described widespread defects in capillary beds and altered red blood cell and platelet production, a cardiovascular system-wide pathology is indicated in SMA.

These systemic pathologies are likely to become increasingly apparent in children treated with Spinraza (Nusinersen), where neuromuscular symptoms are alleviated and life extended, therefore combinatorial therapies to address them must be developed.

NOTES

O10P: IDENTIFICATION AND EVALUATION OF NEW BIOMARKERS FOR SMA – SKELETAL MUSCLE AND MITOCHONDRIAL DEFICITS

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Spinal muscular atrophy (SMA) is a severe genetic disorder that manifests in progressive neuromuscular degeneration. SMA originates from loss-of-function mutations of the SMN1 (survival motor neuron 1) gene. Recent evidence suggests that in SMA not only denervation, but also skeletal muscle deficits and mitochondrial dysfunction significantly contribute to the progression of disease.

In this study, we explore potential biomarkers to measure peripheral deficiencies in SMA patients. We aim to characterize skeletal muscular dysfunctions and mitochondrial deficits in SMA patients in more detail and to identify potential biomarkers quantifying these deficits. Discovery of novel exploratory biomarkers determining skeletal muscular and mitochondrial dysfunctions may allow better assessment of potential therapeutic benefit of investigational medicines like splicing modifiers and mitochondrial protecting compounds on these deficits in clinical trials.

We identified significant differences in five new skeletal muscle biomarkers (GDF-8, sTnI, Fabp3, Myl3, Ckm) in plasma samples from SMA Type III patients compared to age-matched healthy volunteers. These five skeletal muscle biomarkers were compared to the standard clinical chemistry measurements ALT, AST and CK. Correlation analysis of the five skeletal biomarkers with quantitative muscle MRI and motor function measurements (MFM and 6MWT) revealed a significant correlation of GDF-8 with these parameters. An additional analysis of mitochondrial parameters in blood samples of over 100 SMA patients identified reduced circulating mitochondrial DNA levels in SMA Type II and Type III patients.

Our biomarker data suggest that five new skeletal muscle biomarkers (GDF-8, sTnI, Fabp3, Myl3, CKm) and mitochondrial DNA provide additional sensitivity and specificity to standard clinical chemistry measurements, and have the potential to become new exploratory biomarkers to monitor the skeletal muscular and mitochondrial deficits in SMA patients.

NOTES

P16: THE ROLE OF MUSCLE SECRETED PROTEINS IN SPINAL MUSCULAR ATROPHY

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SMA is primarily considered as a motor neuron disease. However, it has been shown that other organs and tissues such as skeletal muscle and heart exhibit intrinsic defects. As one of the most recognized characteristics of SMA is the morphological and functional defect of the neuromuscular junction (NMJ) and since skeletal muscle has been acknowledged as an endocrine organ, we investigate whether muscle-secreted molecules play a role for neuronal survival and function at the NMJ.

By proteomic profiling combining SILAC-AHA-mass spectrometry based analysis, we screened proteins from WT and SMA muscle cells, identifying and quantifying 1845 proteins from muscle secretomes. Refining the data set by peptide intensities resulted in a list of 894 proteins detected in the secretome and 2981 proteins in cell samples. With stringent statistical analysis using peptide intensity rather than protein, we could narrow down the list to eight upregulated and ten downregulated proteins in SMA muscle secretomes. Among them, C1q/tumor necrosis factor-related protein 3 (C1qtnf3, also known as CTRP3) is an interesting candidate for further investigations. CTRP3 was recently shown to be involved in metabolic pathways which are known to be dysregulated in SMA. We independently confirmed a reduced secretion of CTRP3 by Smn knockdown C2C12 cells with Western blots. In addition, we are validating CTRP3 expression in muscle tissue of a severe SMA mouse model. Currently, we are intensively evaluating the downstream pathways of CTRP3 in WT and SMA motor neurons.

The contribution of secreted molecules from diseased muscle cells to motor neuron pathophysiology in neurological disorders, especially in SMA, is poorly investigated. Here, we found very interesting candidates differentially secreted from SMA muscle cells.

This study will help us to uncover the unappreciated mechanisms contributing to NMJ impairment in SMA.

NOTES

P17: UBIQUITIN-LIKE MODIFIER ACTIVATING ENZYME 1 (UBA1) IS A KEY MEDIATOR OF NEUROPATHOLOGICAL CHANGES IN SMA

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It is now well established that ubiquitin homeostasis is altered in SMA and that reduction of ubiquitin-like modifier-activating enzyme 1 (UBA1) is central to this disruption. UBA1 is responsible for activating ubiquitin as the first step in the ubiquitin conjugation process, marking unwanted proteins for degradation by the proteasome. While it is known that therapies targeting UBA1 rescue neuromuscular phenotypes in SMA models, the mechanism by which UBA1 mediates neurodegeneration is unclear. To identify downstream targets of UBA1 critical for UBA1-mediated degeneration, label-free proteomics was performed on HEK-293 cells after overexpression or knockdown of UBA1. Changes in tRNA-synthetase pathways, including proteins such as GARS, were identified as UBA1-dependent and validated in both HEK293 cells and the Taiwanese mouse model of SMA. Interestingly, mutations in GARS cause Charcot Marie Tooth disease type 2D (CMT2D), an axonal neuropathy, in which a disruption to sensory neuron cell fate in dorsal root ganglia has recently been identified. In the Taiwanese mouse model of SMA we identified a phenotype consistent with that in the CMT2D mouse model and showed that disruption to sensory neuron cell fate is dependent on changes in UBA1 in SMA. In conclusion, modulation of UBA1 levels leads to disruption of key cellular pathways, with dysregulation of tRNA-synthetases a prominent feature.

NOTES

P18: RESTORING DISTURBED ENERGY HOMEOSTASIS IN SPINAL MUSCULAR ATROPHY

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Spinal muscular atrophy (SMA) is a neuromuscular disease, characterized by the loss of lower alpha motor neurons, that leads to proximal muscle weakness. The molecular background of SMA is described as autosomal recessive with loss or mutation of the ubiquitously expressed gene called survival of motor neuron 1 (SMN1). The molecular mechanism of SMA is not yet fully characterized. However, since the pathology shows similarities with other neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD), it has been associated with metabolic defects and especially impairments in mitochondrial function. The main source of neuronal energy is glucose and motor neurons depend on mitochondrial oxidative phosphorylation (OxPhos) to cover their high energy demands. SMA motor neurons show hyperexcitability which requires high energy levels. Recent findings show that neurons can adapt to increased energy demands by activating mitochondrial biogenesis or the mobilization of glucose transporter to the plasma membrane for enhanced fuel supply. The mobilization of Glucose transporter to the axonal plasma membrane triggered by activity of the synapses is directed by activity of the adenosine monophosphate (AMP)-activated protein kinase (AMPK). Motor neurons from a SMA mouse model show a disturbed energy homeostasis which is pointed out by a reduced number of functional mitochondria along the axon. Accordingly, ATP levels are reduced by 56% in SMA motor neurons compared to control ones. AMPK serves as a key metabolic sensor of cellular energy homeostasis and is activated by phosphorylation under nutritional stress. In SMA motor neurons AMPK is activated due to the decreased energy level. Currently, we try to restore the energy level by supporting mitochondrial function and glycolysis in SMA motor neurons.

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SESSION 4 - MODIFIERS OF PHENOTYPE

012P: CHP1 REDUCTION AMELIORATES SMA PATHOLOGY BY RESTORING DNM1 HYPERPHOSPHORYLATION AND ENDOCYTOSIS

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Recently, the first SMA drug based on antisense oligonucleotides (ASOs) correcting SMN2 splicing, namely SPINRAZA, has been approved. However, in type I SMA patients the ASO induced elevation of SMN levels may be still insufficient to restore motor neuron (MN) function lifelong. PLS3 and NCALD are two SMN-independent protective modifiers identified in humans and proven to be effective across various SMA animal models. Both, PLS3 overexpression and NCALD downregulation protect against SMA by restoring impaired endocytosis. Nevertheless, the exact mechanism behind this protection is largely unknown.

Here, we identified Calcineurin-like EF-hand protein 1 (CHP1) as a novel PLS3 interacting protein using a yeast-two-hybrid screen. Co-immunoprecipitation and pull-down assays confirmed a direct interaction between CHP1 and PLS3. Although CHP1 is ubiquitously expressed, it is particularly abundant in neurons and specifically present at SMA relevant sites such as MN growth cones and neuromuscular junctions (NMJ). Strikingly, we found elevated CHP1 expression in SMA. Indeed CHP1 downregulation restored axonal outgrowth defects in Smn-depleted NSC34 MN-like cells, SMA zebrafish and murine SMA MNs. Most importantly, subcutaneous injection of a low-dose SMN-ASO in presymptomatic mice doubled the survival of a severely-affected SMA model, whereas additional CHP1 reduction, by a heterozygous Chp1 mutant allele, prolonged survival span by 3.2-fold. Moreover, CHP1 reduction ameliorated electrophysiological defects, NMJ size, NMJ maturation and muscle fibre size compared to low-dose SMN ASO alone. In MN-like cells, Chp1 knockdown almost tripled bulk endocytosis while clathrin-mediated endocytosis remained unaffected. Importantly, CHP1 knockdown restored bulk endocytosis in Smn-depleted cells by elevating Calcineurin phosphatase activity. As CHP1 is the counter-player of Calcineurin which collectively dephosphorylates proteins involved in endocytosis, therefore, it is crucial for synaptic vesicle endocytosis. Congruently, we found marked hyperphosphorylation of Dynamin 1 (DNM1) in SMA MNs, which was restored to control level by the heterozygous Chp1 mutant allele.

Taken together, we show that CHP1 is a novel modifier of SMA pathology that directly interacts with PLS3, and ameliorates SMA phenotypes by improving impaired endocytosis. Most importantly, we demonstrate that CHP1 downregulation is a promising SMN-independent therapeutic target for a combinatorial SMA therapy.

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015P: RNA-SEQ AND MOTIF ANALYSIS OF HUMAN MOTOR NEURONS REVEALS A CRITICAL ROLE OF SMN/SYNCRIP COMPLEX AND MOTIF 7 IN SPINAL MUSCULAR ATROPHY

Federica Rizzo[1], Monica Nizzardo[1], Shikha Vashisht[2], Erika Molteni[2], Valentina Melzi[1], Irene Faravelli[1], Sabrina Salani[1], Monica Bucchia[1], Michela Taiana[1], Giulia Forotti[1], Andreina Bordoni[1], Nereo Bresolin[1], Giacomo Pietro Comi[1], Uberto Pozzoli[2], **Stefania Corti[1]**

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Spinal muscular atrophy (SMA) is a genetic motor neuron disease caused by the reduction of the survival motor neuron (SMN) protein, essential for pre-mRNA processing. The exact pathogenic mechanisms and the reasons of selective motor neurons (MNs) vulnerability are still not completely understood. It could be hypothesized that MNs hold specific features that make them more sensitive to lower amounts of SMN protein. SMN has been demonstrated to possess a key role in RNA splicing, although a thorough analysis of the correlation between SMN defect, specific gene expression/splicing alterations, and the presence of common RNA motif sequences in deregulated genes in human SMA MNs is still lacking.

To address this question, we performed deep RNA sequencing and bioinformatic analyses on MNs derived from induced pluripotent stem cells (iPSCs) of SMA patient and healthy subjects. We detected SMA-specific molecular changes in MNs, including abnormalities in axonal and synaptic genes such as in Neurexin and Synaptotagmin genes families. Motif enrichment analysis of differentially expressed/spliced genes in SMA-MNs revealed the presence of common sequences for RNA binding proteins. Among these, hnRNPQ (SYNCRIP), that interacts with SMN but only full-length isoform, binds several of these key MN genes suggesting that the impairment of SMN(full-length)/hnRNPQ interaction in SMA can be responsible for the observed gene expression/splicing misregulation. Overexpression of hnRPNQ in human SMA iPSCs-derived lines improved MN survival and increased motor axon length, restoring identified candidate downstream targets of RNA-processing dysfunction induced by SMN deficiency.

These data demonstrated that SMN is essential for mRNA processing in association with hnRNPs and identified selective RNA pathways disrupted in SMA that can represent alternative therapeutic targets beside SMN.

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Cariplo grant (2012-0513) and JPND (DAMNPATHS) to SC is gratefully acknowledged

NOTES

P19: MOLECULAR BASIS OF RESPIRATORY FAILURE IN SPINAL MUSCULAR ATROPHY

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Spinal muscular atrophy (SMA) is caused by the chronic low levels of survival motor neuron (SMN) protein and is characterized by motor neuron degeneration and muscle atrophy leading to respiratory distress. Respiratory complications, including bronchial inflammation, pulmonary infections, lung defects such as atelectasis, are the most reported condition in severe SMA patients. Respiratory failure causes death in SMA but the underlying molecular mechanism is unknown. The zinc finger protein ZPR1 interacts with SMN. ZPR1 is down regulated in SMA patients. We report that ZPR1 functions in collaboration with SMN to regulate HoxA5 levels in phrenic motor neurons that control respiration. Spatiotemporal inactivation of Zpr1 gene in motor neurons down-regulates HoxA5 and causes defects in the function of phrenic motor neurons. Phrenic motor neurons fail to arborize in the diaphragm leading to diaphragmatic paralysis that results in respiratory failure and perinatal lethality in mice. In SMA mice, SMN-deficiency causes down-regulation of ZPR1 and HoxA5 that result in degeneration of phrenic motor neurons. Biochemical and molecular analysis show that ZPR1 is a transcription factor that interacts with human HOXA5 promoter and modulation in ZPR1 levels directly correlates and regulates levels of HOXA5 transcription. Identification of ZPR1 and HoxA5 as potential targets provides a paradigm for developing strategies to treat respiratory distress in SMA.

NOTES

P20: DIFFERENTIAL SYNAPTOTAGMIN-1 EXPRESSION COULD BE DETERMINANT FOR MUSCLE VULNERABILITY IN SMA

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Spinal muscular atrophy (SMA) is a genetic disease caused by the loss/mutation of the Survival Motor Neuron (SMN1) gene. The disease is characterized by motor impairment, with axial and proximal limb muscles being more affected than distal muscles.

In the SMNΔ7 SMA mouse model, the motor impairment is characterized by an early and significant reduction of neurotransmitter release in vulnerable muscles. The molecular basis of the functional deficit and its selectivity is not well understood yet.

In this work, we investigated whether one or more synaptic proteins are differentially altered in SMA motor nerve terminals and its possible relationship with the deficit in neurotransmission. To this end, we compared the expression level of different synaptic proteins in control and SMNΔ7 mice, namely synaptic vesicle protein 2 (SV2A, SV2B, SV2C), synaptotagmin (Syt1, Syt2, and Syt7) and syntaxin 1B (Stx1B), in four muscles: two greatly affected, the Transversus abdominis anterior (TVA) and the Obliquus internus abdominis (OIA), and two less affected, the Levator auris longus (LAL) and the diaphragm. Our immunofluorescence and quantitative confocal microscopy results show a direct correlation between the degree of muscle vulnerability and the SV2B and Syt2 level in SMA nerve terminals in comparison with control littermates. Syt1 levels, however, were little affected in mutants compared with controls in the muscles studied. Also, we found that the physiological expression of Syt1 varies among motor terminals of different muscles. For example, at postnatal day 9 (P9), Syt1 is absent or very low in motor nerve terminals of the TVA and OIA, while it is still abundant in nerve terminals of more preserved muscles in SMA such as the diaphragm and LAL. Consequently, more affected muscles in SMA present lower overall levels of synaptotagmin in the presynaptic terminals than the less vulnerable muscles.

In summary, we show that in SMA motor nerve terminals there is a deficit in two key proteins for neurotransmission (Syt2 and SV2B). Our results also show a high physiological expression of Syt1 in nerve terminals of the less vulnerable muscles, suggesting that Syt1 may compensate for the overall decrease of Syt2 and protect the synapse from its early impairment in these muscles.

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P21: IMPAIRMENT OF AXONAL AND DENDRITIC GROWTH IN CRISPR/CAS9-MEDIATED CHONDROLECTIN KNOCKOUT MICE

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BACKGROUND:
Spinal muscular atrophy (SMA) is a neurodegenerative disease caused by deficiency of the SMN protein. A hallmark of SMA is the selective degeneration of spinal motor neurons. In previous work we established that reduced levels of SMN are not associated with a generalised disruption of splicing before disease onset (Baumer, 2009). Differential expression of an isoform of chondrolectin (Chodl), a protein highly expressed in spinal motor neurons (MNs), was identified as an early event in SMA disease pathogenesis, and experimental manipulation of Chodl has distinct effects on cell survival and neurite outgrowth in MNs in a zebrafish model of SMA (Sleigh, 2014).

RESULTS:
Here, we have utilized a CRISPR/Cas9 mediated chondrolectin knockout mouse model to investigate the function of Chodl in motor neurons by characterizing the in vitro phenotype. Analysis of embryonic MNs from this mouse model revealed differences in axon and dendrite length compared to wild-type MNs, with primary motor neuron cultures from knockout mice showing truncated axonal processes. Further studies will involve crossing this mouse with milder SMA mouse models to look for further evidence that Chodl can affect the SMA phenotype. Our ongoing work is focussed on performing a comprehensive neuropathological analysis and behavioural phenotyping of this mouse model.

CONCLUSIONS:
Collectively, these results suggest that chondrolectin depletion results in neurite and axon defects in primary cultures and confirm a potential role for this protein in neuromuscular development.

ACKNOWLEDGEMENTS:
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NOTES

P22: IDENTIFICATION AND CHARACTERIZATION OF SPINAL MUSCULAR ATROPHY MODIFIERS

Svenja Schneider[1,2,3], Vanessa Grysko[1,2,3], Natalia Mendoza-Ferreira[1,2,3], Anna Kaczmarek[1,2,3], Markus Riessland[4], Ilja Bobylev[2,5], Laura Torres-Benito[1,2,3], Eva Janzen[1,2,3], Heiko Loehr[2,6,7], Frank W. Rigo[8], C. Frank Bennett[8], Kathryn J. Swoboda[9], Matthias Hammerschmidt[2,6,7], Brunhilde Wirth[1,2,3,10]

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SMA is caused by ubiquitous SMN deficiency leading to progressive degeneration of α-motoneurons in anterior horns of the spinal cord. In a large discordant SMA family we identified five asymptomatic and two SMA affected individuals all carrying homozygous SMN1 deletions. Transcriptome and linkage analysis led to the identification of a novel SMA protective modifier,_Neurocalcin delta (NCALD), which was significantly downregulated in asymptomatic compared to symptomatic or control individuals. NCALD downregulation was further confirmed to be beneficial in vitro and in vivo as it ameliorated or rescued several SMA pathologies by restoring clathrin-mediated endocytosis. Nevertheless, the beneficial effect of NCALD knockdown in a severe SMA mouse model was rather subtle, most likely due to severe impairments of peripheral organs causing premature death. In order to mimic the situation observed in human asymptomatic individuals, a milder SMA mouse model was established following two strategies: (I) breeding SMA mice on the more robust mixed genetic background (mixed50) and (II) subcutaneous injection of a single low-dose SMN ASO (30 µg) that increases correct SMN2 splicing leading to moderately elevated SMN levels. These mice did not exhibit peripheral organ impairments and survived over 180 days, yet they showed neuronal and motoric impairments resembling the milder SMA symptoms in humans. We demonstrated that heterozygous Ncald knockout in this mild SMA mouse model improved neuromuscular junction size and maturation, enhanced motoric abilities, and moderately increased nerve conduction capacities. Together, these findings suggest a novel therapeutic option for SMA combining the SMN ASO with additional NCALD downregulation to counteract disease symptoms as in the asymptomatic individuals from the SMA discordant family.

NCALD belongs to a conserved protein family of neuronal calcium sensors (NCS). We started to investigate another NCS protein family member, neuronal calcium sensor 1 (NCS1), on the SMA background. NCS1 downregulation rescued neurite/axon outgrowth defects caused by SMN loss both in vitro in neuron-like cells and in vivo in an SMA zebrafish model. Thus, NCS1 can be considered as a potential novel SMA modifier.

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P23: EVALUATION OF POTENTIAL EFFECTS OF PLS3 AND SMN-ASO ON PUTATIVE BIOMARKERS IN SPINAL MUSCULAR ATROPHY MICE

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Spinal muscular atrophy (SMA) is a recessive neuromuscular disorder caused by homozygous loss of the survival of motor neuron 1 (SMN1) gene. SMN2, a nearly identical copy gene, produces only about 10 % of full-length SMN2 transcript and its copy number inversely correlates with the severity of SMA. Nusinersen, a SMN antisense oligonucleotide (ASO), blocks an intronic silencer in SMN2 pre-mRNA and facilitates full-length SMN2 splicing. Recently, Nusinersen has been FDA and EMA-approved for SMA therapy. Plastin 3 is a modifier of SMA identified in humans and its rescuing effect is proven in a broad range of SMA animal models. PLS3 is able to rescue axonal growth defects and has beneficial effects on the neuromuscular junction maturation and function. ASOs and genetic modifiers provide encouraging perspectives for the treatment of SMA. However, the effects of them on the identified putative biomarkers are still elusive. Furthermore, the effect of Nusinersen and modifier genes on the plasma level of putative biomarkers is still not clear. The aim of this study was to analyse the feasibility of seven putative SMA biomarkers which have been described to be correlated with motor function in SMA patients. We measured the levels of SMN in whole blood via an ECL immunoassay and the level of six SMA biomarkers, namely Cartilage Oligomeric Matrix Protein (COMP), Dipeptidyl Peptidase 4 (DPP4), Tetranectin (C-type Lectin Family 3 Member B, CLEC3B), Vitronectin (VTN), Fetuin A (Alpha 2-HS Glycoprotein, AHSG) and Osteopontin (Secreted Phosphoprotein 1, SPP1) in plasma from SMA and HET mice, SMA and HET mice overexpressing PLS3 from a transgene, and wild type mice – each genotype treated with low-dose SMN-ASOs or maintained untreated. Mice were analysed at two time points: P10 and P21. The data were analysed with regard to the stability of the biomarkers over time, the possibility to discriminate different disease severities, the potential influence of PLS3 on known SMA biomarkers and compared our results to recent literature data in humans and mice. SMN and COMP were the most promising biomarkers. Plasma protein levels differed significantly in SMA mice compared to the controls at both time points. Furthermore, our results showed that low-dose SMN-ASO treatment or PLS3 overexpression does not change the level of SMN or COMP in blood, supporting the hypothesis that PLS3 acts protective independent of the SMN pathway.

NOTES

P24: AUTOPHAGY INHIBITION DELAYS MOTOR NEURON DEGENERATION AND DISEASE PROGRESSION IN A MURINE MODEL OF INTERMEDIATE SMA

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Spinal Muscular Atrophy (SMA), a recessive autosomal neuromuscular disease, is characterized by motor impairment, muscle atrophy and premature death following motor neuron (MN) degeneration, due to the lack of SMN (survival motor neuron) protein. Emerging evidence suggests that dysregulation of autophagy can contribute to MN degeneration.

Here we have investigated the role of autophagy in the SMNdelta7 mouse model of SMA II (intermediate form of the disease) which leads to motor impairment by postnatal day 5 (P5) and to death by P13.

First of all we have showed by immunoblots that Beclin 1 and LC3-II expression levels increased in the lumbar spinal cord of the SMA pups. Electron microscopy and immunofluorescence studies confirmed that autophagic markers were enhanced in the ventral horns of SMA mice. To clarify the role of autophagy, we administered intracerebroventricularly (at P3) either an autophagy inhibitor (3-methyladenine, 3-MA), or an autophagy inducer (rapamycin) in SMA pups. Motor behavior was assessed daily with different tests: tail suspension, righting reflex and hindlimb suspension tests. 3-MA significantly improved motor performance, extended the lifespan and delayed MN death in lumbar spinal cord compared to control-group. Inhibition of autophagy by 3-MA suppressed autophagosome formation, reduced the apoptotic activation (cleaved caspase-3 and Bcl2) and the appearance of TUNEL-positive neurons, underlining that apoptosis and autophagy pathways are intricately intertwined.

Therefore, autophagy is likely involved in MN death in SMA II, suggesting that it might represent a promising therapeutic target for delaying the progression of SMA in humans as well, possibly in combination with the emerging promising treatments aimed at manipulating the SMN expression.

NOTES

P25: TARGETING RNA STRUCTURE IN SMN2 REVERSES SPINAL MUSCULAR ATROPHY MOLECULAR PHENOTYPES

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Modification of SMN2 exon 7 (E7) splicing_is a validated therapeutic strategy against the neuromuscular disease Spinal Muscular Atrophy (SMA). However, a target-based_approach to identify small molecule E7 splicing modifiers has not been attempted, which could reveal novel therapies with improved mechanistic insight. Here we chose as a target the stem-loop RNA structure TSL2, which overlaps with the E7 5' splicing site (5' ss). A small molecule TSL2-binding hit, natural compound homocarbonyltopsentin (PK4C9), was identified that increased E7 splicing to therapeutic levels and rescued downstream molecular alterations in SMA cells. High-resolution NMR combined with in silico modeling, RNA sequencing, and mutagenesis studies revealed that PK4C9 binds to the pentaloop conformation of TSL2 and promotes a shift to a triloop conformation that displays enhanced E7 splicing efficiency. Collectively, our study validates TSL2 as a target for small molecule drug discovery in SMA, identifies a novel mechanism of action for an E7 splicing modifier, and sets a precedent for other splicing-mediated diseases where an RNA structure could be similarly targeted.

NOTES

P26: MOTOR NEURON SPROUTING AS A PHENOTYPIC MODIFIER IN 'RESISTANT' MUSCLES AND MICE

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A hallmark of SMA is denervation of muscles. However, not all muscles appear to be equally affected. In the Smn2B/- mouse model of SMA, abdominal musculature displays vast denervation, while cranial musculature remains relatively spared, even at a late stage of the disease. The reasons for this remain unknown. In addition, there are mouse models that have reduced Smn but are asymptomatic. In particular, the Smn2B/2B mouse model has 30% of normal Smn levels yet shows no phenotypic onset of disease. Mounting evidence suggests that sprouting plays a part in masking a phenotype in these resistant muscles and mice. We have therefore investigated this concept. We have quantified degenerative and regenerative features at the NMJ of Smn2B/- and Smn2B/2B mice in various muscle groups. This includes endplate occupancy, neurofilament accumulation, endplate size and the presence of different types of neuronal sprouts. In the Smn2B/- mouse model, sprouting appears to correlate with the differential vulnerability of muscles. In the Smn2B/2B mouse model, data gathered is suggesting that sprouting may not be masking a phenotype and rather there may be some other process providing NMJ stability or indeed Smn levels within this model are sufficient to maintain motor neuron survival.

NOTES

P27: UNDERSTANDING THE THERAPEUTIC TIME WINDOW IN SMA

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Over recent years there have been multiple therapeutic strategies for SMA in various stages of development and clinical trials, but it remains to be seen how efficient these therapies can be when they are given at different stages throughout disease progression. Recent studies have suggested that there is a therapeutic window, outside of which therapies will fail to significantly improve motor function and extend life span. The identification of a therapeutic time window presents further questions: how severe is the phenotype at a point when a full recovery is possible? How well does the neuromuscular system recover if a therapy is provided within this window?

Utilising the SmnRES model, a cre-mediated rescuable mouse model of SMA, we have analysed the severity of the neuromuscular junction (NMJ) phenotype at an early time point associated with a full recovery. Interestingly, we report a significant decrease in endplate size and occupancy, and a significant increase in neurofilament accumulation. This level of NMJ pathology is surprising given the capacity for a full recovery. We go on to restore SMN levels at this early time point and reanalyse the NMJ phenotype post-recovery. We report an increase in endplate occupancy and a decrease in neurofilament accumulation in our rescued mice when compared with SMA mice.

These results provide a promising insight in to the efficacy of SMA therapies, and provide an ideal platform to explore combinational therapies.

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P28: INHIBITION OF MYOSTATIN ACTIVATION BY SRK-015 PROMOTES MUSCLE STRENGTH IN A MULTIPLE MOUSE MODELS OF SMA

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Pharmacological inhibition of myostatin is a promising therapy for many muscle diseases. Multiple anti-myostatin therapies are currently in the clinic, many of which also inhibit related family members, such as GDF11 and Activin A. This lack of selectivity has the potential to result in unwanted side effects, some of which may be particularly important to avoid in pediatric populations.

Myostatin is expressed as an inactive proprotein and undergoes two cleavage steps to release and activate the mature growth factor. While the mature form of myostatin is highly homologous to other TGFβ family members, most notably GDF11, their pro-domains are very divergent. We therefore targeted the pro-form of myostatin to generate highly specific antibodies that prevent release from the prodomains. One such antibody, SRK-015, inhibits the second cleavage step, preventing activation of mature myostatin.

We have confirmed that SRK-015 specifically binds pro- and latent myostatin and does not recognize mature myostatin or any forms of GDF11 or Activin A. We have also shown that SRK-015 increases muscle mass and force in healthy mice and prevents muscle loss in a dexamethasone-induced model of atrophy.

Here we demonstrate that the parental clone of SRK-015, SRK-015P, improves muscle function in multiple models of SMA. We first assessed the ability of SRK-015P to increase muscle function in two variants of the Δ7 model. The first variant aimed to approximate type II SMA: Δ7mice were administered a subtherapeutic dose of the SMN splice modulator SMN-C1 from birth until day 24, after which the dose was increased to a high, therapeutic dose, and SRK-015P treatment initiated. The second variant aimed to model type III/IV SMA: Δ7 mice are given high dose SMN-C1 from birth, with SRK-015P treatment again beginning at day 24. In both models 4 weeks of SRK-015P treatment resulted in significant improvements in muscle force. Additionally, we observed significant increases in cortical and trabecular bone volume in Δ7 mice administered high dose SMN-C1 from birth and 4 weeks of SRK-015P treatment.

The data presented here indicate that blocking myostatin activation with SRK-015 has therapeutic

potential for SMA, both as a monotherapy and as an adjunct to splice modulator therapies. In addition, the specificity of this antibody for myostatin may be of particular relevance for safety in a chronic treatment setting for pediatric populations.

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SESSION 5 - PRE-CLINICAL COMBINED THERAPIES

O16P: COMBINATORIAL ASO THERAPY USING SMN-DEPENDENT AND SMN-INDEPENDENT PROTECTION - NCALD REDUCTION - AGAINST SMA

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Spinal muscular atrophy (SMA) is a neuromuscular disease with an incidence of 1:6000 to 1:10,000 and the most frequent genetic cause of childhood lethality. Recently, SPINRAZA, an antisense oligonucleotide (ASO) that corrects SMN2 splicing and thus increases full-length SMN protein has been FDA- and EMA-approved for SMA therapy. However, the administration of ASOs in very severe and/or post-symptomatic patients might be insufficient to counteract the disease. We believe that additional SMN-independent therapies may be required especially in type I SMA, where only two SMN2 copies are available; and/or in all types of SMA to support motorneurons (MN) and neuromuscular junction (NMJ) function after disease onset. We identified PLS3 and NCALD as protective modifiers of SMA in humans, while their modifying role has been proven in various animal models of SMA including worm, flies, zebrafish and mice. Despite PLS3 overexpression is the stronger protective modifier, NCALD downregulation seems easier to be targeted by ASOs and to be used in combination with SMN ASOs.

Based on the encouraging results from genetically modified mice, we developed in collaboration with IONIS Pharmaceuticals Ncald ASOs to reduce NCALD level. From the initial 30 Ncald ASOs, which were generated and tested in cells and adult mice, we chose the three most efficient ones to be tested in neonatal mice. We optimized them for tolerability and efficiency in the Taiwanese SMA mouse model on mixed background (50%C57BL6/N; 50%FVB/N) developed in our lab. Ncald3 ASO showed the optimal viability, with non-toxic effects and the best decrease of the protein expression: 75% in brain and 80% in spinal cord.

To analyse the impact of reduced NCALD on the SMA phenotype, we next performed a preclinical study using presymptomatic injection of low-dose SMN+Ncald ASOs compared to SMN+control ASOs, where Ncald- or control ASOs were injected ICV at P2 and SMN ASOs subcutaneously at P1. Preliminary results showed a significant increase in CMAP (compound muscle action potential) in SMA mice injected with SMN+Ncald ASOs compared to SMN+control ASOs at P21. A future perspective of the present project is to validate the combinatorial therapy at different ages (3 and 6 months) and in postsymptomatic stages and to analyse these mice in detail (motoric abilities, MN, NMJ and muscle development).

NOTES

017P: TARGETING THE 5'UTR OF SURVIVAL MOTOR NEURON 2 (SMN2) TO INCREASE ITS EXPRESSION IN A DISEASE MODEL OF SPINAL MUSCULAR ATROPHY

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SMN2 is capable of producing full-length SMN protein, but does so at a relatively low rate due to exon 7 skipping in a majority of transcripts. Increasing the total number of SMN2 transcripts has the potential to compensate for loss of SMN1. The 5' and 3' untranslated regions (UTRs) of a gene contain cis-regulatory elements that modulate transcript stability and/or translational efficiency. We sought to determine whether the 5'UTR of SMN2 contains a repressive feature that limits its expression, targeting of which could increase SMN levels. Splice-switching oligonucleotides, such as nusinersen, have been successfully used to increase full-length SMN levels, but their effects are limited by the abundance of SMN2 transcripts in a cell. Thus, we further asked if targeting the 5'UTR and exon 7 splicing simultaneously could enhance SMN expression more than targeting either region alone. We identified an antisense oligonucleotide (ASO) complementary to the 5'UTR of SMN2 that increases SMN protein levels in SMA patient fibroblasts. Further, this ASO significantly increases SMN2 mRNA levels, but not pre-mRNA levels, suggesting the ASO offers protection to mature transcripts. Interestingly, 5'UTR ASO treatment shifts the SMN2 isoform ratio toward exon 7 inclusion, possibly through a feedback loop involving the SMN protein itself, which has a well-characterized role in spliceosome biogenesis. Combining the 5'UTR ASO with a previously developed splice-switching oligonucleotide results in a further increase in SMN protein levels. Future experiments will examine the mechanism of action of this ASO. Our results add to our current understanding of SMN regulation and may reveal a new therapeutic target for SMA.

NOTES

018P: DYSREGULATED SIGNALING IN SMA: FROM ISOLATED PATHWAY APPROACHES TO A CLUSTERED NETWORK REPRESENTATION

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Spinal Muscular Atrophy is caused by low levels of functional SMN protein. However, downstream disease mechanisms remain elusive. In the recent years several studies suggested a number of signaling pathways which mediate pathological changes in SMA. We identified molecular mechanisms linking SMN with altered profilin/ROCK signaling as well as with an enhanced ERK-activity. Moreover, we showed a connection between both pathways since ROCK inhibits ERK and vice versa. Co-inhibition experiments in SMA-mice demonstrated that this lateral connection between both signaling axes is relevant for the SMA-like pathophysiology. This indicates that an isolated pathway approach may be a rather reductionistic model of dysregulated signaling in SMA. Here, we employed a screening against phospho-proteins in pre-symptomatic and symptomatic SMA-mice which allows us to identify several dysregulated targets simultaneously. A bioinformatic analysis identified three clustered networks, growth factor signaling, MAPK signaling, and cell cycle / DNA repair which are interconnected. Identification of key-signaling nodes within each of these clusters is an important milestone which allows a rescue of SMA-like phenotypes alone or in combined drug approaches.

Acknowledgements
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NOTES

019P: IMPROVED IN VITRO MODELS OF THE HUMAN BLOOD-BRAIN BARRIER (BBB) USING ENDOTHELIAL CELLS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS (IPSCS) FOR TESTING CNS THERAPEUTICS

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The blood-brain barrier (BBB) is primarily composed of highly specialised brain microvascular endothelial cells (BMECs), pericytes and end processes of astrocytes. The BBB tightly controls the exchange of molecules and cells between the brain and the blood. Although the BBB successfully maintains the brain microenvironment, it also blocks beneficial therapeutics for diseases of the central nervous system (CNS). The tight junction between the BMECs is the recognised phenotype of the BBB and is empirically defined by high trans-endothelial electrical resistance (TEER) and low permeability to paracellular markers. Models derived from animal tissue have proven useful, but suffer from relatively low TEER values and high permeability. We have produced improved in vitro models of the BBB using BMECs differentiated from human induced pluripotent stem cells (iPSCs). Three different clones of wild-type (4603, 19-9-7T and AD3-CL1) and a single clone of Spinal Muscular Atrophy type I (SMA I) iPSCs were differentiated into BMECs, characterised and assessed for proficiency to form BBB models. The integrity of the models was evaluated using TEER, expression of tight junction protein occludin, and permeability to paracellular markers lucifer yellow (LY) and sodium fluorescein (NaF). For comparison, the TEER of the most widely used in vitro model of the human BBB, made of the primary human brain endothelial cell line hCMEC/D3 (D3), was used.

The TEER values of 4603 and 19-9-7T derived BMECs are comparable to the value reported for co-culture models using endothelial cells, pericytes and astrocytes and over 60-fold higher than D3 cells. The SMA I iPSC-derived BMECs have higher TEER than AD3-CL1 iPSC-derived BMECs and 40-fold higher than D3 cells. The permeability of iPSC-derived BBB models to LY and NaF was 40-fold and 3-fold less than in the D3 model, respectively. Seven-fold more 4603 BMECs express glucose transporter-1 (GLUT-1) compared to D3 cells. All iPSC-derived BMECs express tight-junction protein occludin, whereas D3 cells do not. Our in vitro models of the BBB with BMECs alone display tight junction that closely mimics the human BBB in vivo and will have many potential uses including testing of therapeutic agents aimed at the CNS and investigating BBB breakdown in disease states. We are currently testing the crossing of therapeutics for SMA through our BBB models.

This work was funded by The SMA Trust through the UK SMA Research Consortium.

NOTES

P29: THE SIGMA-1 RECEPTOR AGONIST PRE-084 ATTENUATES REACTIVE GLIOSIS AND PREVENTS MOTONEURON DEAFFERENTATION IN MOUSE MODELS OF SPINAL MUSCULAR ATROPHY

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Spinal muscular atrophy (SMA) is a devastating genetic disease characterized by loss of motoneurons (Mns), skeletal muscle atrophy and paralysis. SMA is caused by deletion or inactivating mutations of the survival motor neuron 1 (SMN1) gene resulting in deficient SMN protein levels. The most promising strategies for therapy are those aiming to enhance SMN expression by using antisense oligonucleotides, and gene therapies to directly replace SMN1 gene. However, SMN-independent, complementary therapies aimed to ameliorate and/or preserve neuromuscular system integrity and function are also necessary. Excitability properties of MNs appear to play a critical role in their degree of vulnerability. MN excitability is modulated by cholinergic inputs mediated by C-type synapses (C-boutons). The Sigma-1 receptor (Sig1R) is a protein highly expressed in MNs, particularly located at C-boutons. Sig1R has a pleiotropic role in MNs and appears to be involved in the modulation of excitability. Previous studies have demonstrated that Sig1R activation prevents MN death, preserves motor activity and prolongs survival in mouse models of amyotrophic lateral sclerosis (ALS). We explored here whether treatment with the Sig1R agonist 2-(4-morpholinethyl) 1-phenylcyclohexanecarboxylate (PRE-084) was able to exert beneficial effects in spinal muscular atrophy (SMA). Two murine models of SMA were used: the SMNΔ7 (severe model) and Smn2B/- (intermediate model) mice. We report here that chronic administration of PRE-084 attenuates reactive gliosis and restores the microglial phenotype (M1/M2) balance altered by the disease and, consequently, increases the beneficial anti-inflammatory phenotype of these cells. Moreover, the Sig1R agonist partially prevents the loss of afferent inputs on SMA MNs. Nevertheless, PRE-084 does not elicit positive effects on median survival, motor abilities, MN degeneration, and major histopathological changes in neuromuscular junctions of SMA mice.
Supported by the Spanish Ministerio de Economía y Competitividad (SAF2015-70801-R).

NOTES

P30: A SPECIFIC IPSC-DERIVED NEURAL STEM CELLS SUBPOPULATION, POSITIVE FOR LEWISX, CXCR4 AND B1 INTEGRIN, IMPROVES THE PATHOLOGY OF A MOUSE MODEL OF SMARD1

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Spinal muscular atrophy with respiratory distress type I (SMARD1) is an infantile autosomal recessive genetic disease caused by mutations in the IGHMBP2 gene without a cure. A promising therapeutic strategy is the transplantation of neural stem cells (NSCs) derived from induced pluripotent stem cells (iPSCs), that ameliorate, even if not completely rescue, the phenotype of SMARD1 animal model nmd, by protecting their endogenous motor neurons (Simone et al., 2014). We reported that a specific NSC subpopulation expressing the stem cell marker LewisX, CXCR4 and β1 integrin, useful for cell migration, was therapeutically advantageous relative to unselected NSCs in terms of delivery and engraftment (Nizzardo et al., 2016). In this study we tested the therapeutic potential of this selected NSC fraction in the context of SMARD1.

Wild-type iPSCs have been differentiated into NSCs and selected for the expression of the specific molecules LeX-CXCR4-β1. To investigate their therapeutic potential, we administered intrathecally the NSCs selected fraction into SMARD1 mice at postnatal day 1. We verified that after injection, LeX+CXCR4+β1 NSCs properly migrated from the central nervous system and engrafted in the spinal cord parenchyma, particularly in the anterior horns. In the nmd mice, engraftment of the selected NSC subpopulation resulted in significant improvement in the phenotypic hallmarks of the disease. Transplanted nmd mice showed ameliorate overall appearance, recovery of neuromuscular functions and increase in lifespan. The specific NSC subpopulation protected SMARD1 endogenous motor neurons from degeneration and, besides exerted a positive effect on the cell soma, it also had a beneficial effect at the periphery, promoting neural muscular junction maintenance and collateral axonal sprouting. Moreover, NSC LeX+CXCR4+β1+ NSC treatment positively affects muscular tissues, that presented an ameliorated muscle fiber morphology and organization and less fatty infiltration.

Overall, we demonstrated that transplantation of a specific human iPSC-derived NSC fraction, selected to enhance migration, survival and engraftment, has a beneficial role in modifying the course of SMARD1 pathology protecting endogenous motor neurons from degeneration. Our results confirm the potential role of NSC transplant for cell-mediated therapy in SMARD1.

NOTES

P31: THE EXERCISE MIMETIC AGENT AICAR PREVENTS NEUROMUSCULAR PATHOLOGY BUT DOES NOT MITIGATE CLINICAL DETERIORATION IN A MOUSE MODEL OF SPINAL MUSCULAR ATROPHY

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It has been reported that physical exercise is beneficial in spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS) by improving motor function. The AMPK agonist AICAR is an exercise mimetic agent that has been shown to ameliorate the dystrophic muscle phenotype in the mdx mouse, a model of Duchenne muscular dystrophy. Here we investigated whether chronic AICAR administration was able to elicit beneficial effects in a mouse model of severe SMA (the SMNΔ7 mouse), in a similar way as reported for physical exercise. SMNΔ7 and WT mice were used. AICAR (500 mg/Kg/day) or vehicle was daily administered from postnatal day 1. Tests to assess motor behavior were performed, and muscles and spinal cords were obtained for analysis. We found that AICAR improved muscle atrophy and increased the proportion of type I skeletal myofibers in SMNΔ7 animals. Moreover, AICAR reduced axonal sprouting, mitigated denervation and favored maturation of SMA neuromuscular junctions. Although AICAR prevented the loss of glutamatergic excitatory synapses on MNs, it was not able to reduce MN degeneration or the microglial and astroglial reaction found in the spinal cord of diseased mice. No improvement in motor abilities and lifespan was seen in SMNΔ7 animals subjected to AICAR treatment. AICAR did not induce changes in the expression of SMN protein in the spinal cord and skeletal muscles, indicating that beneficial effects of the compound in SMA are SMN-independent. These results indicate that AICAR is an effective agent for promoting muscle trophism in SMA, and can be a potential therapeutic option to be used synergically with other MN-directed strategies in the disease.

Supported by the Ministerio de Economía y Competitividad (SAF2015-70801-R).

NOTES

P32: A STEM CELL MODEL OF SPINAL MUSCULAR ATROPHY (SMA): TARGETING A COMBINATORIAL DRUG THERAPY

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Spinal Muscular Atrophy (SMA) is one of the most common juvenile neurodegenerative diseases. The disease is caused by a mutation of ubiquitously expressed gene, Survival Motor Neuron 1 (SMN1), leading to reduce SMN protein. The significant death noticed in neuronal population, particularly motor neurons in SMA is either due to developmental maturation error or death of motor neurons over time. Using primary cultures and induced pluripotent stem cells (iPSC) as a model system, we noticed significant morphological changes such as reduced cell size and the axonal length. In addition to that the nuclear condensation is one of major phenotype notice in these cells. Elevated levels of Caspase3 in motor neurons derived from iPSCs clearly demonstrating the role of apoptosis in SMA pathology and therapies targeting this cascade may have significant clinical applications. Our aim is to concentrate on non-SMN mediated therapeutic strategies and combinatorial drug treatment to provide additional protection to neuronal population and evade muscle degeneration in SMA. We will use different signaling molecules that are targeting motor neuron quality control combined with co-SMN to widen the scope of developmental medicine in SMA.

NOTES

P33: DOSE COMPARISON OF MORPHOLINO ANTISENSE OLIGONUCLEOTIDES IN SEVERE AND STANDARD TAIWANESE SMA MICE

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The human SMN2 transgenic mouse, or "Taiwanese" mouse model, possesses phenotypes which mimic human patients with Type I spinal muscular atrophy (SMA). Previous studies have shown dose-dependent therapeutic effects of the morpholino antisense oligonucleotide, PMO25, on various physiological scores of the model, such as body weight, mobility, muscle pathology and survival. In this study, two different doses of PMO25 were tested in a colony of Taiwanese SMA mice with a severe phenotype, and one dose tested in a standard Taiwanese SMA mouse colony. Results showed that the median survival time in the severe model extended only modestly from 8 days in untreated SMA mice to 12.5 days in SMA mice receiving 10 mg/g of PMO25 subcutaneously at day of birth or postnatal day 1 (P1, n=4). A higher dose at 20 mg/g did not further extend the median survival time, but one long-lived mouse survived to P26 and developed a necrotic tail. In the standard Taiwanese model, 20 mg/g of PMO25 greatly increased the median survival time to 47 days (n=10), compared to 11 days in untreated mice (n=15), as described previously. Righting times were not distinguishable between control and PMO25-treated SMA mice. Furthermore, as early as at P46, some PMO25-treated SMA mice started to develop necrotic ears and tail, phenotypes which have previously been observed in mice with milder forms of SMA. This study confirms that PMO25 shows significant therapeutic effects in the standard Taiwanese SMA mouse model, but also demonstrates that treatment outcomes in more severely affected mice are compromised.

NOTES

Session 6: EMERGING PHENOTYPES AND STANDARDS OF CARE

O20P: MRI OF THE CERVICAL SPINAL CORD AND NERVE ROOTS IN SMA

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OBJECTIVE:
What we know of SMA pathology is mainly based on post-mortem and animal studies. The early detection of changes in pathological mechanisms in SMA patients in vivo is increasingly important in light of therapeutic developments. Innovative MRI techniques could help to dissect pathological mechanisms and provide unique anatomical and functional biomarkers of disease progression or response to treatment. Therefore we assessed the feasibility of novel MRI protocols in SMA patients and healthy controls.

METHODS:
We developed 4 MRI protocols of the cervical spinal cord and nerve roots of spinal segments C3-C8 on a 3 Tesla MRI system. Two anatomical MRI protocols to investigate 1) cross-sectional area (CSA) at each spinal segment of the whole spinal cord and grey and white matter separately (Fig B) and 2) the diameter of anterior and posterior nerves at each spinal segment (Fig A) and two functional diffusion tensor imaging (DTI) protocols to assess the diffusion parameters fractional anisotropy (FA), mean (MD), axial (AD) and radial diffusivity (RD) of 1) segments C5-C7 of the cervical spinal cord (Fig C) and 2) cervical nerve roots (C3-C8) (Fig D). For all 4 protocols we investigated the difference in measures between patients and healthy controls.

RESULTS:
We included 10 patients with SMA types 2-3 and 20 age-and gender-matched healthy controls. We found an overall smaller CSA in patients compared to controls ($p=0.017$), with atrophy rates up to 8.5% at spinal segment C7. This difference seems mainly caused by a decrease in grey matter CSA. DTI data showed a slightly higher FA in the grey matter of patients compared to controls ($p=0.032$). Mean thickness of anterior nerves overall (C4-C8) was 0.93±0.13 mm in patients and 1.05±0.27 mm in controls. Thickness of posterior nerves was 1.03±0.22 in patients and 1.12±0.35 mm in controls. In the nerve roots (C3-C8) all DTI parameters were lower in patients compared to controls, but significant differences in MD, AD and RD were located at the rostral segments C3-C5 ($p<0.037$).

CONCLUSION:
We found differences between patients and controls using both structural and functional MRI protocols, confirming the potential of this technique to assess pathological mechanisms in SMA. When further developed and evaluated longitudinally in a larger group, it could provide novel biomarkers that can be implemented in therapy development and evaluation.

NOTES

021P: END OF STUDY RESULTS FROM ENDEAR: PROPORTIONS OF HINE-2 AND CHOP INTEND RESPONDERS

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BACKGROUND:
ENDEAR (NCT02193074) was a phase 3, randomized, double-blind, sham-procedure controlled 13-month study of the efficacy and safety of nusinersen in infantile-onset spinal muscular atrophy (SMA).

OBJECTIVE:
To report the likelihood of motor function responses among nusinersen-treated and control infants who were alive and remained in the ENDEAR study.

METHODS:
Symptomatic infants diagnosed with SMA (most likely to develop SMA Type I) were randomized (2:1) to receive intrathecal nusinersen (12-mg scaled equivalent dose) or sham-procedure. Eligibility criteria included: genetic diagnosis of 5q SMA, 2 SMN2 copies, age ≤6 months at symptom onset, and age ≤7 months with no hypoxemia at screening. The first primary endpoint was the proportion of Hammersmith Infant Neurological Examination section 2 (HINE-2) motor milestone responders excluding voluntary grasp, defined as those with (1) ≥2-point increase or maximal score in kicking ability or ≥1-point increase in head control, rolling, sitting, crawling, standing, or walking and (2) more HINE categories improving than worsening. A secondary endpoint was the proportion of Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) responders, defined as those with a ≥4-point improvement from baseline in CHOP INTEND score. Infants who died or withdrew from the study were not included in the current analyses.

RESULTS:
Of nusinersen- and sham procedure-treated infants alive at each time point, the likelihood of HINE-2 response was 51% (30/59) versus 9% (2/23) at Day 183, 61% (22/36) versus 0% (0/16) at Day 302, and 77% (20/26) versus 0% (0/11) at Day 394. When the later of Day 183, 302, or 394 assessments were used for each infant with a ≥Day 183 assessment, the overall likelihood of HINE-2 response was 64% (37/58) in nusinersen-treated infants versus 0% (0/20) in control infants. The likelihood of CHOP INTEND response followed a similar pattern with rates increasing over time in the nusinersen treatment group. Among nusinersen- and sham procedure-treated infants who were alive at each time point, 90% (52/58) versus 5% (1/20) were CHOP INTEND responders when the later of Day 183, 302, or 394 assessments was analyzed.

CONCLUSION:
The likelihood of response on motor function assessments increased over time on study. Most nusinersen-treated infants who were alive and remained in the study were responders on the HINE-2 (64%) and CHOP INTEND (90%) at their last study visit.

NOTES

022P: COGNITIVE DEVELOPMENT, LANGUAGE AND USE OF AUGMENTATIVE ALTERNATIVE COMMUNICATION IN SMA1 CHILDREN IN ITALY

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Today "technology dependent" SMA1 patients, with either invasive or non-invasive respiratory support, may reach their teens. It is known that extreme muscular weakness prevents SMA1 children from speaking clearly. Despite their vocalizations and glances, children are not always able to express what they think and this may make them angry and/or frustrated, even if there are many differences between one child to another. This is a very important issue because it affects the wholeness of the person. Nevertheless, the improvement of Communication is still not universally considered within Standards of Care.

The AAC PEPE-which is short for Augmentative Alternative Communication Program for Early Parental Empowerment, was developed for SMA1 patients in order to guarantee a model of AAC intervention targeted at the characteristics of SMA1. Early exposure to AAC as an input is in fact especially important for SMA1 children to experiment possible functions and to support internal language and experiences.

We decided to test fifteen children with SMA1 aged 3.8–11.2 years. One-dimensional Raven test (CPM) was used to evaluate cognitive development, and Brown Bellugy modified for Italian standards (TCGB) was used to evaluate language comprehension.

All 15 children collaborated to CPM, with an average IQ of 116. Eight children collaborated to TCGB, that was in the normal range in all of them. Children with an early AAC intervention scored in the higher range in both tests.
In 2009: 3 SMA1 children were involved in AAC PEPE
In 2017: 55 SMA1 children, 25 with early onset between 6 and 9 months, are involved in the project.
Young children are immediately exposed to listening to books with full text in symbols (Inbook), and by 3 years of age they are able to use advanced technological tools, can communicate independently and their communication is effective and rewarding.
We would like to point out how early investment in AAC influences abilities in: cognitive, linguistic, interaction, relationship, thought, active participation in daily life, decision-making, learning.

Thanks to the AAC project, the quality of life is improved of both SMA1 children and their families resulting in the acquisition of:

- higher IQ and better language comprehension
- extended interpersonal, communication skills
- stronger identity, greater independence
- increased self-esteem, self-efficiency
- development of cognitive potential in mainstream classes
- can drive power wheelchair with switches.

NOTES

023P: CLINICAL CHALLENGES IN THE TREATMENT OF SPINAL MUSCULAR ATROPHY (SMA) WITH NUSINERSEN

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In June 2017, the European Medicines Agency approved the antisense oligonucleotide (ASO) nusinersen for the treatment of all for all 5q-associated spinal muscular atrophy (SMA) types. The 2´-O-methoxyethyl phosphorothioate modified antisense drug nusinersen has to be administered intrathecally within an initial saturation period (day 0, 14, 28 and 63) to warrant drug saturation followed by a frequent maintaining application in 4 month intervals. Due to the clinical features of SMA, the clinical application of the ASO by lumbar punction is clinically challenging considering the frequently observed scoliosis, previous orthopedic surgeries or the need of sedation especially in infants or young children.

We are currently treating 19 patients with 5q-associated SMA (type 1 to 3) in the age ranging from 8 months up to 52 years, 7 with permanent or intermittent ventilatory support. We report from clinical observations and challenges regarding the treatment procedures that often require interdisciplinary collaborations (neurologists, neuroradiologists, pediatrics, orthopedics and anesthesiologists). Here, CT-graphic supported lumbar punction is the most challenging issue regarding drug application and was necessary in 8 of the patients. In detail, 4 of these 8 patients had prior lumbar spine surgery (e.g. spondylodesis) whereas a severe scoliosis was clinically evident in the other 4 patients that lead to frustrating lumbar punction without imaging support. Further, in infants and children, topical anesthetics and opioids (e.g. nalbuphine) in moderate dosage had to be administered to warrant successful lumbar punction. Indeed, adolescents and adults received solely topical and local anesthetics for lumbar punction with the exception of long-lasting punction procedures where patients had to be treated with benzodiazepines such as midazolame, opioids or anesthetics such as s-ketamine.

However, adolescent or adult SMA-patients with less severe disease progression are currently also under treatment with nusinersen in our department but the available therapy monitoring strategies and outcome variables regarding clinical efficacy such as motor scales seem not be appropriate considering that main motor functions have been clinically developed. Thus, we suggest further clinical and laboratory features to monitor the clinical efficacy especially in older and those with less disease progression such as cerebral spinal fluid, imaging and electrophysiological parameters.

NOTES

024P: BENEFITS OF EARLIER TREATMENT WITH NUSINERSEN IN INFANTS AND CHILDREN WITH SPINAL MUSCULAR ATROPHY (SMA)

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BACKGROUND: Nusinersen has shown significant and clinically meaningful efficacy on motor milestone achievement and motor function in multiple SMA populations, on survival in infantile-onset SMA and a favourable safety profile.

OBJECTIVE: To evaluate the benefits of nusinersen based on age and disease duration at the time of screening in symptomatic children and infants who started treatment in a pre-symptomatic stage.

DESIGN/METHODS: ENDEAR and CHERISH were randomised, double-blind, sham procedure-controlled studies of nusinersen in infantile-onset SMA (most likely to develop SMA Type I) and later-onset SMA (most likely to develop SMA Type II or III), respectively. NURTURE is an ongoing, single-arm, open-label study of nusinersen in infants in a pre-symptomatic stage of SMA (most likely to develop SMA Type I or II). All study participants had documented 5q SMA. ENDEAR enrolled symptomatic infants age ≤7 months with onset of SMA at age ≤6 months and 2 SMN2 gene copies. CHERISH enrolled symptomatic children age 2–12 years with onset of SMA at age >6 months who had sat but never walked independently. NURTURE enrolled infants age ≤6 weeks and no clinical signs of SMA at first dose with 2 or 3 SMN2 gene copies.

RESULTS: In ENDEAR, higher proportions of HINE motor milestone responders were observed with nusinersen compared with sham control in the overall population and in subgroup analyses based on disease duration at screening and age at SMA onset (≤12, >12 weeks; Fig). In CHERISH, HFMSE LSM scores were significantly improved over 15 months with nusinersen versus control (Fig). Greater benefits were observed in younger children and those treated sooner after symptom onset (Fig). Similar results were noted for other ENDEAR and CHERISH endpoints. Mean HINE total motor milestone scores increased over time in nusinersen-treated infants in NURTURE. Infants with 2 SMN2 gene copies treated in the pre-symptomatic stage in NURTURE had higher mean HINE total motor milestone scores after ~1 year of treatment (mean[SE] score at Day 365: 19.7[1.7]) compared with ENDEAR infants who initiated treatment after symptom onset (mean[SE] score at Day 394: 7.19[1.0]).

CONCLUSIONS: Nusinersen showed consistent benefits on measures of motor function in infantile-onset SMA, later-onset SMA and in infants in a pre-symptomatic stage. Subgroup analyses indicate that initiation of nusinersen in infants/children with SMA earlier in their disease course maximises the treatment benefit.

NOTES

P34: EVALUATION OF CHILDREN WITH SPINAL MUSCULAR ATROPHY TYPE 1 DURING THE EXPANDED ACCESS PROGRAM IN GERMANY

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Nusinersen is the first approved drug to treat Spinal Muscular Atrophy (SMA). It acts as splicing modifier of the SMN2 gene upregulating levels of functional SMN protein. Prior to approval, Nusinersen was available to patients with SMA type 1 in Germany within an Expanded Access Program (EAP). In contrast to the previous phase I-III clinical trials, children with SMA type 1 of different age groups and different stages of the disease were treated with Nusinersen within the EAP.

We performed a prospective data collection of patients with SMA type 1 treated with Nusinersen within the EAP. Data were collected during routine patient visits on standardized case report forms. Nusinersen was administered intrathecally on treatment days 1, 15, 30, 60 and 180. We collected data regarding respiratory, gastrointestinal or orthopedic symptoms and monitored adverse events. Furthermore, we used the CHOP INTEND and motor milestones on treatment days 1, 60 and 180 as functional outcome measures. Physiotherapists were trained for the CHOP INTEND in organized workshops.

We collected data from 58 patients with SMA type 1 in 6 different neuropediatric departments in Germany. Age at initiation of treatment ranged between 2 and 96 months (median 17 months).

Within the EAP, Nusinersen was available to children with SMA type 1 of all different age groups and is now, after approval, provided to patients with all different types of SMA. Thus, a broader spectrum of patients can be treated with Nusinersen, but partially without any previous experience from clinical trials. Due to improvements in treatment and technological advances, the natural history of SMA patients has changed. To improve the care of patients with SMA, a comprehensive and systematic collection of data regarding the natural history of the disease and the influence of drug treatments is crucial. Therefore, we will conduct a prospective, multicenter non-randomized registry in German-speaking regions called SMARTCARE with the aim to collect and evaluate data from all SMA patients irrespective of their actual treatment regime.

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Acknowledgement: This work is funded by Initiative SMA

NOTES

P35: NATURAL HISTORY OF SPINAL MUSCULAR ATROPHY: A LONG-TERM PROSPECTIVE SINGLE-CENTER POPULATION BASED COHORT STUDY IN THE NETHERLANDS

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BACKGROUND
The different spinal muscular atrophy (SMA) phenotypes are all characterized by a decline in motor abilities over time but with different rates of progression. For the most common and severe type of SMA (type 1), progression is fast and life-expectancy is short. The natural history of 'classic' SMA type 1 has been studied extensively in the past decade and provided valuable background information for clinical trial design and assessment of therapy efficacy.
For SMA types 2–4, disease progression is slower but can nevertheless cause significant progressive functional impairment. Its natural history has not been studied as extensively as in SMA type 1: large cohort studies with long-term follow-up data that reflect the natural courses of SMA types 2–4 are relatively scarce. This, to some degree, causes uncertainty regarding different aspects of the natural course of the disease, e.g. specific rates of decline of motor function in different age groups (i.e. young children, older children, adolescents, adults).
Therefore, natural history studies remain important: their data help to further improve our understanding of the disease and its different phenotypes, as well as provide valuable input for outcome measures and clinical trial design.

RESULTS
In 2010 we started a prospective, population-based cohort study of SMA in The Netherlands. In 2015 we added follow-up at regular intervals to our protocol to further document the natural history of the disease, with a keen interest in SMA types 2–4, given the relative scarcity of studies on the natural history of these patients. Approximately 300 Dutch patients with SMA have been registered and included in our single center cohort study, of whom until now approximately 100 patients have returned for follow-up visits (45% type 2; 48% type 3).
Clinical data on (the decline in) motor abilities, including muscle strength (MRC), the expanded Hammersmith Motor Function Scale (HMFSE), the Motor Function Measure (MFM), and CHOP INTEND, as well as laboratory data, including electrophysiological tests (EMG), SMN protein, and molecular biomarkers, are collected at every visit.

CONCLUSIONS
We are performing a prospective follow-up study of all SMA types in a clinically and genetically well-defined cohort of patients. Up-to-date preliminary results of our work will be presented during the conference.

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NOTES

P36: SPINAL MUSCULAR ATROPHY: WHAT HAVE WE LEARNED ABOUT THE EYE FROM EXPERIMENTAL STUDIES? POTENTIAL CLINICAL IMPLICATIONS

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Spinal muscular atrophy (SMA) is caused by a loss-of-function defect in the SMN1 gene, leading to reduced levels of the survival of motor neuron (SMN) protein. The disease is characterised by a loss of motor neurons from the anterior horn of spinal cord and atrophy of skeletal musculature. Type I SMA is the most severe form with an onset either in utero or immediately postnatally.

As SMN protein is ubiquitously-expressed, we used the two keywords: “eye” and “SMA” (PubMed) for searching papers about eye lesions in SMA. Only two experimental studies were identified.

According to Liu et Al. (2010), retinas of the Smn-depleted mice have altered electroretinograms. In Smn 2B/- mice (an intermediate model of SMA), they observed reduction in the axon number and glial cell number within the optic nerve, compromised axodendritic outgrowth in the retina, and a loss of retinal neurons.

In the other hand, the gene encoding NAIP (neuronal apoptosis inhibitory protein) is frequently deleted in type I SMA. Ingram-Crooks et al (2002) reported NAIP gene transcript levels during murine embryogenesis within various developing tissues and organs. They observed NAIP transcription in the spinal cord of mouse embryo between days 9.5 and 14.5 of pregnancy. At day 16.5, NAIP transcripts were found in the retina of mouse embryos._

In embryogenesis, the neural retina of a mammalian eye is derived from the forebrain neuroepithelium and remains later connected to the brain via the optic nerve.

SMN protein is present in neural retina (retinal ganglion cells and amacrine cells) as well as in glial cells in the optic nerve. The eye is therefore an excellent model for studying neuronal development and pathogenesis of central nervous system disorders. Moreover, ganglion cells in the retina and motor neurons in the spinal cord share some developmental and functional characteristics (long axon, axon pathfinding mechanisms).

Despite the absence of reports focused on the eye in SMA patients, early-onset retinal lesions are very likely present, at least in type I SMA infants. In the context of the lengthening of life expectancy, and a better quality of life in SMA patients, a systematic screening of retina using ERG and/or OCT (optical coherence tomography) could provide new insights into SMA pathogenesis. Retina may be a significant biomarker of SMA progression, and a valuable criteria of efficacity for future clinical trials evaluation.

NOTES

P37: PROGRAM FOR EARLY PARENTAL EMPOWERMENT (PEPE): 30 YEARS' EXPERIENCE IN THE MANAGEMENT OF SMA TYPE I CHILDREN

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The parents of SMA type I child need to learn vertically integrated healthcare competences to manage the disease from a 360° perspective. The Program for Early Parental Empowerment (PEPE) has 30 years' experience (1987-2017) on 484 type I (figure, left panel) SMA children. It aims to identify the best range of care and strategies (knowledge and skills) and to transfer them to the parents. PEPE training allows parents/caregivers to manage their child right from the start at home, to recognize the signs of the disease, to deal with daily activity and to handle urgencies/emergencies. PEPE training starts with the use of: 1) suction machine, 2) bag valve mask, 3) oximeter, 4) nasogastric feeding tube and 5) massage and postural training. These are the required procedures in case of palliative care when parents opt to follow the natural history of SMA to prevent their child from a technology-dependent life. When parents choose the interventional treatment approach, a proactive management with more intensive motor, respiratory and nutritional care is mandatory to maintain the good health status of the child, to face the new challenges emerging from a growing child and to minimize inappropriate hospital admissions. Parents/caregivers need to learn the use of additional devices and to develop new skills like: 6) cough-assisted device, 7) mechanical ventilation, 8) percutaneous endoscopic gastrostomy, 9) prevention of the deformities, 10) cognitive and communication program and 11) resolution of the contingencies of daily living1. Since 2016, a new drug is available for SMA. The proactive management of a type I child under drug requires increased efforts by the therapists to enhance the new motor skills derived from the drug and ultimately to give force to it. These abilities comprise the development of 12) active motor physiotherapy, and 13) dynamic orthosis aimed to exploit and recruit residual and new muscular functions. PEPE sustains and trains parents/caregivers in all the 13 points (figure, right panel) in order to maintain a relatively high level of wellness for their child, with the awareness and the competence to recognize and manage any acute episodes, while respecting the progression of the disease and to promote the new motor skills resulting from the new treatments.

1Finkel RS et al. Neuromuscul Disord. 2017 Jun;27(6):596-605

NOTES

P38: DEVELOPMENT OF A PREDICTIVE ENERGY EQUATION FOR SPINAL MUSCULAR ATROPHY TYPE I CHILDREN

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BACKGROUND:
A clear understanding of energy need in Spinal Muscular Atrophy type I children (SMAI) is an important issue that has significant clinical implications to provide optimal nutrition care and to avoid the frequently reported defective or excessive derangement of nutritional status.

OBJECTIVE:
The study objectives were to explore the predictors of measured resting energy expenditure by indirect calorimetry (mREE) among a sample of SMAI, to generate a predictive energy equation and to compare such models to that suggested by General Nutrition Guidelines for Spinal Muscular Atrophy (SMAGNG).

DESIGN AND METHODS:
The study was a cross-sectional cohort design conducted at the International Center for the Assessment of Nutritional status (ICANS), University of Milan. Study subjects were SMAI children (N = 79) recruited from 2 clinical referral centers for SMA in Italy (Developmental Neurology Unit, Carlo Besta Neurological Institute Foundation, Milan, and S.A.PRE., Early Abilitation Service, Mangiagalli e Regina Elena Hospital, Milan). Demographic [sex and age (months)], anthropometric [Body Weight (BW,kg) and Supine Length (SL,cm)], body composition [total Fat Free Mass (FFM, kg) by Dual x-ray Energy Absorptiometry], ventilation status [(spontaneous vs noninvasive mechanical ventilation or mechanical ventilation via tracheostomy] data were examined as potential predictors of mREE.

RESULTS:
The median age of the sample was 10 (6;30) months. 36 children (45,5%) were males. Age, BW, SL, FFM and ventilation status were all significantly ($p<0.05$) correlated with mREE. After screening for multi-collinearity, the best predictive model (SMAICANS) of mREE included BW and ventilation status ($R(2) = 0.66$, $p<0.01$).
Using Bland-Altman plots, the SMAICANS model over- and underpredicted mREE less often than the SMAGNG model.

CONCLUSIONS:
SMAICANS model including BW and ventilation status data explained more than 50% variance of mREE and had better precision in determining energy requirements for SMAI patients when compared with SMAGNG. Further research is necessary to improve predictive models of mREE in the SMA population and to test its validity and clinical application.

NOTES

P39: THE EFFECT OF PERCUTANEOUS ENDOSCOPIC GASTROSTOMY ON DIETARY INTAKE, NUTRITIONAL STATUS AND BODY COMPOSITION IN CHILDREN WITH TYPE I SPINAL MUSCULAR ATROPHY

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BACKGROUND&AIM:
Percutaneous endoscopic gastrostomy (PEG) placement is frequently performed in children with type I spinal muscular atrophy (SMAI), to overstep swallowing dysfunction, to reduce gastroesophageal reflux and to reverse growth failure and undernutrition. However, long term outcome on dietary intake and nutritional status after PEG placement has been poorly investigated. To this purpose, the aim of this study was to investigate the effect of PEG feeding on energy and macronutrients daily intake and on anthropometrics and body composition parameters in SMAI children.

METHODS:
We carried out an observational longitudinal study on 7 SMAI children (mean age: 32±23 months; 4 females). Resting Energy Expenditure (REE, kcal/die) by Indirect Calorimetry, mean daily dietary intake by 3- days food records, Body Weight (BW,kg) and_ Supine Length (SL,cm) by anthropometric measurements and body composition [total and segmental Fat Mass (FM, kg) and Lean Mass (LM, kg)] by Dual x-ray Energy Absorptiometry were collected before and after PEG placement.

RESULTS:
In average, 538±228 days have gone by the first to the second evaluation. Before PEG placement, difference between daily energy intake and measured REE was 262±178 kcal/die. After PEG placement, children showed a significant increment in energy intake which consisted of an increment of both protein, fat and carbohydrates daily mean amount intakes. In particular, after PEG placement, protein intake was equal to twice the Italian recommendations, while fat and carbohydrates intake remained in the reference range. Concerning nutritional status and body composition, anthropometric measurements did not change after PEG placement compared baseline, but legs FM significantly increased with concomitant legs LM decrement (table below).

CONCLUSION:
With PEG feeding, the difference between daily energy intake and resting energy expenditure almost doubled and it was due to highest daily intake of all macronutrients, in particular proteins. This increase was not supported by an improvement of nutritional status; at most, it seemed to get worse legs fat mass and lean mass distribution of lower limbs as usually occur in severe hypomobility. An accurate evaluation of resting energy expenditure after PEG placement is recommended to provide an adequate nutritional intervention to improve and not worsen nutritional status of SMAI children.

NOTES

P40: SCOLIOSIS IS AN INESCAPABLE COMORBIDITY IN SMA TYPE II. A SINGLE CENTER EXPERIENCE

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BACKGROUND:
Scoliosis is the most debilitating and unresolved problem in SMA type II patients.

PATIENTS AND METHODS:
We present data of progression of scoliosis and treatment in a cohort of 20 SMA type II patients followed in the last fifteen years (2002-2017) in our Neuromuscular Unit. Medical records were collected prospectively. In all patients brace was prescribed since Cobb angle in sitting position was ≥20° or in case of marked kyphosis. All patients underwent systematical X-Ray, functional and pulmonary assessments.

RESULTS:
Seventeen of 20 patients (age range 2-20 y) have scoliotic curve. Mean age at scoliosis development was 3,4 y (range 2-7 y) and Cobb angle at diagnosis varied from 5° to 35°. Nine patients underwent scoliosis surgery. Six patients had Growing-rods or VEPTR at mean age of 8,1 y (range 6,2- 10y). Five patients (two of them received at first growing-rods) underwent definitive spine fusion at a mean age of 12.2 y (range 10-14y). Postoperative complications were observed in 3 patients: urinary infection, difficulty in extubation and weaning from mechanical ventilation and infective complication of the surgical wound.

CONCLUSIONS:
Despite the use of brace, scoliosis progressed in all cases and, as observed in our series, since the age of 6 years patients develop severe rotoscoliosis that, following the current standards of care, requires surgery. These findings have to be carefully considered in the developing of potentially effective therapy intrathecally administered.

NOTES

P41: NUSINERSEN DECREASES THE INCIDENCE AND LENGTH OF HOSPITALIZATIONS IN INFANTS WITH SPINAL MUSCULAR ATROPHY: RESULTS FROM THE ENDEAR STUDY

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BACKGROUND:
_ ENDEAR (NCT02193074) was a phase 3, randomized, double-blind, sham-procedure controlled 13-month efficacy/safety study of nusinersen in infants with SMA (most likely to develop SMA Type I).

OBJECTIVE:
_ To assess the number and length of hospitalizations experienced by infants who participated in the ENDEAR study.

DESIGN/METHODS:
_Symptomatic infants diagnosed with SMA were randomized (2:1) to receive intrathecal nusinersen (12-mg scaled dose) or sham-procedure. Eligibility criteria included genetic diagnosis of 5q SMA, age ≤6 months at symptom onset, 2 SMN2 copies, and age ≤7 months with 02 saturation ≥96% at screening. Number and length of hospitalizations were tertiary endpoints. Hospitalization was defined as an admission of >24 hours to a medical facility. The number of hospitalizations was summarized using the rate at which they occurred to account for the differing numbers of infants and lengths of observations. The aggregate hospitalization rate per group was calculated by dividing the total number of hospitalizations by the total number of subject-years on study. The adjusted annualized hospitalization rate (AAHR) was based on a negative binomial regression adjusted for symptom onset age and disease duration at screening. Hospitalization length was calculated as the mean proportion of time spent hospitalized, reported as least square means (LSM) based on analysis of covariance adjusted for symptom onset age and disease duration at screening.

RESULTS:
80 infants (age: 32-210 days) received nusinersen and 41 (age: 20-211 days) received sham procedure; 73 and 37, respectively, were included in this analysis. The AAHR (95% CI) was 4.378 (3.636, 5.273) with nusinersen and 5.817 (4.441, 7.620) with control (rate ratio: 0.753 [95% CI:0.539, 1.052]; P=0.0959). The numbers of hospitalizations were 254 and 113 in the nusinersen and control groups, respectively; the proportions categorized for general observation (3% vs 5%), observation after dosing (21% vs 15%), serious adverse events (71% vs 71%) and ancillary procedures (5% vs 9%) were similar. The overall LSM proportion of time spent hospitalized was lower in the nusinersen than the control group (0.119 vs 0.199) with a LSM treatment difference (95% CI) of -0.080 (-0.141, -0.019; P=0.0104) favoring nusinersen.

CONCLUSIONS:
Nusinersen-treated infants in the ENDEAR study tended to be hospitalized less frequently and spent less time hospitalized than sham-procedure control-treated infants.

NOTES

P42: MR IMAGING OF MUSCLE IN SMA AS A BIOMARKER FOR DISEASE PROGRESSION. PROTOCOL DESIGN FOR AN OBSERVATIONAL COHORT STUDY WITH 1 YEAR FOLLOW-UP

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BACKGROUND
Recently developed treatment strategies for hereditary proximal spinal muscular atrophy (SMA) have further increased the urgency for sensitive clinical outcome measures. Biomarkers that are able to capture treatment efficacy or disease progression before it is reflected by a deterioration of muscle strength or motor function are required.

AIMS
To investigate the value of Dixon for fat fraction, T2 mapping for oedematous alterations and diffusion tensor imaging (DTI) for structural alterations influencing the diffusion properties, as quantitative magnetic resonance imaging (MRI) biomarkers for assessing SMA severity and monitoring disease progression.

METHODS / PATIENTS
Study population: We aim to include 30 patients with SMA types 2-3 and 30 age-matched healthy controls, aged 12 years and over. SMA patients will be recruited from the Dutch SMA database of the SMA Center of Expertise at the University Medical Centre of Utrecht, The Netherlands. The recruitment of healthy controls will go through the initiative of patients. Exclusion criteria consist of any type of (non-)invasive ventilation, severe swallowing disorders, >15% decrease in FVC due to postural change from sitting to supine, or any contra-indication for 3-Tesla MRI.
Study design: A 150 mm image stack (FOV 480x240 mm2) of both upper legs will be acquired on a 3-Tesla MRI scanner (Philips Ingenia) of all patients and healthy controls. The MRI protocol is designed as a fast and clinically feasible protocol; the duration is < 10 minutes and consists of 1) Dixon Imaging, 2) a T2 mapping sequence and 3) diffusion weighted images.
All patients will undergo motor function assessment. Muscle strength will be assessed in all participants using both manual and quantitative testing of selected muscles.
Follow-up interval is 1 year for SMA patients and for controls between 12 and 18 years.
Structural and functional changes will be assessed in relation to clinical characteristics, genetic factors and clinical scores.

RESULTS
The MRI protocol has been tested on healthy controls for the upper leg.
Recruitment has started. Patients will be included until 2019.
So far, all MR examinations were successfully completed and well tolerated by patients and controls.

CONCLUSION
We aim to investigate the biomarker potential of various MRI techniques and to gain further insight into the pathogenic mechanisms of SMA.

NOTES

P43: PARENTS' ADVICE TO HEALTHCARE PROFESSIONALS WORKING WITH CHILDREN WHO HAVE SPINAL MUSCULAR ATROPHY

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AIM:
To explore parents' advice to healthcare professionals working with children with spinal muscular atrophy (SMA).

MATERIALS AND METHODS:
This study derives from a Swedish nationwide survey and uses content analysis to make inferences from answers to an open-ended question concerning parent's advice to healthcare professionals. Of eligible parents who had a child born in Sweden between 2000 and 2010, diagnosed with SMA type 1 or 2, and for whom respiratory support was considered in the first year of life, 61 participated in the study (response rate: 87%). Of these, 51 parents answered the question about advice to healthcare professionals working with children with SMA.

RESULTS:
More than half of the advice from parents was related to professional–family relations. The second most frequent type of advice related to two aspects of knowledge about SMA: desire that healthcare professionals possess knowledge, and desire that they provide knowledge. The parents also had advice concerning support in daily life, both to the parents and to the affected child. Other pieces of advice were related to organization of care and the parents' desire to be involved in the child's care.

CONCLUSIONS:
Parents advised healthcare professionals to increase their disease-specific knowledge, to treat the parents as experts on their child, and to treat the family with respect, particularly in situations where the child's case is used as an opportunity to improve healthcare professionals' competence. Increased practical support in daily life and a case coordinator is also among parents' advice to healthcare professionals.

NOTES

P44: RANGE OF EXTENSION IN THE NEUTRAL POSITION AND ABDUCTION OF THE HIP JOINT IN SMA INDIVIDUALS – A NEW ASPECT OF SUPPORTED STANDING PRACTICE

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INTRODUCTION:
One of the factors determining a proper body position while standing is an appropriate range of extension in hip joints (HE). The authors' experience shows that during the measurement of the range of HE in SMA individuals, an examined lower limb is often naturally abducted.
The aim of the study was to compare the values of HE in a neutral position, i.e. parallel to the longitudinal body axis, and in a preferred position of a limb in the coronal plane, and to assess correlation between the time of support standing and HE in SMA individuals. The other goal was to compare values of HE in a neutral position obtained in SMA and control groups.

MATERIAL AND METHODS:
Individuals aged 2-21 years with SMA type 1, 2 and 3 were qualified to the study. The control group included healthy children and youth aged 2-18. The measurements of HE in SMA individuals were made in a supine position with Rippstein Plurimeter, both in a neutral position and in an individual preferred limb position. Information regarding the duration and quality of the supported standing of SMA patients has been collected.

RESULTS:
75 SMA individuals (SMA1-18, SMA2 -39, SMA3 -18) aged 2-21years (6.1±4.2) were qualified for the study. The control group included 202 healthy individuals aged 2-18 years (8.5±3.2).
In 60 (80%) SMA participants lower limbs were abducted during the examination of HE. Mean values of HE in a neutral position in SMA group were significantly lower than in abduction ($p<0.05$). The values of HE obtained in SMA1 and SMA2 individuals were lower than in SMA3 group ($p<0.01$). HE differed depending on age. A significant correlation was noted between the duration of supported standing practice and the range of extension in hip joints in a neutral ($R=-0.312$, $p=0.001$) and preferred position ($R=-0.259$, $p=0.008$). In individuals who were standing for a longer time, milder contractures in hip joints were noted.The values of HE in both hips in SMA1, SMA2, SMA3 groups were significantly lower than in the control group ($p<0.01$).

CONCLUSIONS:
HE in SMA patients depends on the position of limb in the coronal plane. Abduction increases the range of HE. Supported standing practice in a slight abduction in hip joints should be considered in SMA patients. Longer supported standing facilitates maintaining a larger HE in hip joints.

NOTES

SESSION 7 - CHALLENGES OF CLINICAL TRIALS AND BEYOND

025P: CLINICAL EFFECTS OF NUSINERSEN INJECTIONS IN SMA TYPE 1 PATIENTS OLDER THAN 7 MONTHS: 10 MONTHS OF FOLLOW UP

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OBJECTIVE:
To evaluate safety, tolerability and clinical efficacy of nusinersen treatment in SMA type 1 patients older than 7 months.

INTRODUCTION:
Nusinersen is the first market approved treatment for spinal muscular atrophy. Clinical trial in SMA type 1 involved only patients younger than 7 months, therefore data are lacking in older patients, in which safety and efficacy have not been studied so far.

METHODS:
Since December 2016, we have started the treatment by nusinersen in 35 SMA type 1 patients older than 7 months (16 females and 19 males). Patients have been evaluated at pre-treatment, at two months of treatment and every 4 months subsequently.

We performed neurological examination and HINE (Hammersmith Infant Neurological Examination) motor milestones score. We noted weight gain, ventilatory and nutritional support, hospitalizations and side effects of the treatment. According to their age, patients were assessed by an experienced physiotherapist in a scale with CHOP Intend (Children's Hospital of Philadelphia), MFM (Motor Function Measure) 20 or 32, HFMS (Hammersmith Functional Motor Scale). In few cases, formal evaluation was not performed due to limited mobility or poor cooperation of patients.

RESULTS:
The median age at first injection was 24.5 months [8.3 – 113]. Median follow-up was 10.4 months [7.0 – 12.85]. 14 patients had 2 copies of SMN2, 19 patients had 3 copies and in 2 patients the number of copies was not quantified. Up to date, nusinersen was safe and well tolerated in our cohort. Patients presented with variable but significant and constant motor improvement. In 5 cases, sitting position was acquired. 12 patients improved by more than 2 points their HINE score. Patients' and family's quality of life was improved according to parents' declarations.
Conclusions: Results in this cohort of older patients are in line with data obtained in younger patients during the phase 3 study (ENDEAR). More longitudinal data are mandatory to determine long-term benefit and cost-effectiveness of the treatment.

NOTES

027P: FIREFISH, A MULTI-CENTER, OPEN-LABEL TRIAL TO INVESTIGATE THE SAFETY AND EFFICACY OF Rg7916 IN BABIES WITH TYPE 1 SMA: STUDY UPDATE AND REAL-LIFE EXPERIENCE OF STUDY IMPLEMENTATION

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SMA is caused by mutation or deletion of the survival of motor neuron 1 (SMN1) gene; a second SMN gene, SMN2, produces low levels of functional SMN protein. RG7916 is an oral, small-molecule SMN2 pre-mRNA splicing modifier that distributes into CNS and peripheral tissues and increases SMN protein levels.

FIREFISH (NCT02913482) is a multi-center, open-label, seamless pivotal study evaluating the safety and efficacy of RG7916 in babies aged 1–7 months at enrollment with Type 1 SMA and two SMN2 gene copies. The exploratory Part 1 (n=8–24) is assessing the safety, tolerability, pharmacokinetics and pharmacodynamics of RG7916 at different dose levels. In Part 1, patients receive RG7916 for at least 4 weeks (or 2 weeks after steady-state is achieved) of daily administration; patients then enter an extension phase with RG7916. The confirmatory Part 2 (n=40) will assess the safety and efficacy of RG7916 at the dose level selected from Part 1 over 24 months. The primary endpoint for Part 2 is the proportion of infants sitting without support for 5 seconds, assessed by the Gross Motor Scale of the BSID-III, after 12 months of treatment. As planned in the study protocol, a Safety Monitoring Committee regularly reviews all safety information from all FIREFISH participants.

At the time of abstract submission, no drug-related adverse events leading to study discontinuation have been observed in any patients with SMA receiving RG7916. In addition to a FIREFISH study update, real-life experiences and insights into conducting such a study in Type 1 SMA will be presented. These include coordinating a multi-disciplinary team of healthcare specialists dealing with such young babies, relocating families away from their home country and the importance of assuring standard-of-care practices whilst patients participate in the trial.

The FIREFISH study is ongoing and currently open for recruitment globally.

FIREFISH is sponsored by F. Hoffmann-La Roche.

NOTES

028P: A LONG-TERM, OPEN-LABEL FOLLOW-UP STUDY OF OLESOXIME IN PATIENTS WITH TYPE 2 OR NON-AMBULATORY TYPE 3 SPINAL MUSCULAR ATROPHY WHO PARTICIPATED IN A PLACEBO-CONTROLLED PHASE 2 TRIAL

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Olesoxime is an oral, daily administered compound that supports the function of mitochondria. In a previous randomized, double-blind Phase 2 study (NCT01302600) in patients aged 3–25 years with Type 2 or non-ambulatory Type 3 SMA, olesoxime maintained motor function over 24 months, whilst the placebo group declined. OLEOS (NCT02628743) is an open-label extension study (OLEOS; NCT02628743) assessing the long-term safety and efficacy of olesoxime in patients with Type 2 or non-ambulatory Type 3 spinal muscular atrophy (SMA).

One hundred and twenty-nine patients with Type 2 or non-ambulatory Type 3 SMA from previous Phase 2 study were enrolled and treated with olesoxime (10 mg/kg); the majority have been followed for 12 months (n=104). Primary endpoint is safety and secondary endpoints include change in Motor Function Measure (MFM) D1+D2 from baseline up to 5 years. OLEOS baseline visit occurred 2.4–5.1 years (median 3 years) after study drug discontinuation in Phase 2.
Consistent with previous studies, olesoxime was generally safe and well tolerated at the dose assessed. Maintenance of motor function observed over 2 years in the Phase 2 study was followed by a substantial decline in MFM D1+D2 (>2 points/year) after drug discontinuation. However, the ~2-point MFM treatment difference between olesoxime and placebo at the end of Phase 2 was maintained at OLEOS baseline. Furthermore, olesoxime open-label treatment stabilized motor function (mean change in MFM D1 + D2 from baseline: 6 months, -0.03 [SD, 4.79; n=124]; 12 months, -0.22 [SD, 4.74, n=104]). These data support the long-term stabilization of motor function observed in the Phase 2 study. A study update will be presented.

These data suggest that olesoxime offers the potential to provide meaningful clinical benefit and may play a role in the future therapeutic management of SMA.
Study sponsored by F. Hoffmann-La Roche

NOTES

P45: PRELIMINARY EVIDENCE FOR PHARMACODYNAMICS EFFECTS OF RG7916 IN JEWELFISH, A STUDY IN PATIENTS WITH SPINAL MUSCULAR ATROPHY WHO PREVIOUSLY PARTICIPATED IN A STUDY WITH ANOTHER SMN2-SPLICING TARGETING THERAPY

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Spinal muscular atrophy (SMA) is a rare hereditary neuromuscular disease caused by loss-of-function of the SMN1 gene. While SMN1 produces full-length SMN protein, a second gene, SMN2, produces low levels of functional SMN protein due to alternative splicing of SMN2 pre-mRNA. RG7916 is an orally administered, centrally and peripherally distributed SMN2 pre-mRNA splicing modifier that increases SMN protein levels. JEWELFISH (NCT03032172) is an exploratory, open-label study to establish the safety, tolerability and pharmacokinetics (PK) of RG7916 in patients who have previously participated in a study with a therapy targeting survival of motor neuron 2 (SMN2) mRNA splicing.

JEWELFISH is a multicenter, open-label, exploratory study evaluating the safety, tolerability and PK of daily oral RG7916 in patients with SMA Type 2 or 3, age 12–60 years, who previously participated in a study with therapies targeting SMN2 splicing. The pharmacodynamic (PD) effects on SMN2 mRNA and SMN protein are also assessed.

At the time of abstract submission, 8 patients had received RG7916 for up to 32 weeks. To date, RG7916 has been safe and well tolerated, with no drug-related safety findings leading to withdrawal in any SMA patient exposed to RG7916. Preliminary PD data following RG7916 treatment showed a rapid increase in the full-length SMN2 (SMN2FL)/SMNΔ7 mRNA ratio after treatment onset, and an up to 4-fold increase from baseline over 4 weeks. This SMN2FL/SMNΔ7 mRNA ratio increase resulted in an up to 4-fold SMN protein increase over 4 weeks. A JEWELFISH update including safety and available biomarker data will be presented.

JEWELFISH is currently recruiting in sites across Europe and the US. Together with the ongoing SUNFISH (SMA Type 2 and 3) and FIREFISH (SMA Type 1) studies, JEWELFISH will provide valuable insights into the safety, tolerability, PK and PD of daily oral Rg7916.

Study sponsored by F. Hoffmann-La Roche

NOTES

P46: UPDATED PHARMACODYNAMIC AND SAFETY DATA FROM SUNFISH PART 1, A STUDY EVALUATING THE ORAL SMN2 SPLICING MODIFIER RG7916 IN PATIENTS WITH TYPE 2 OR 3 SPINAL MUSCULAR ATROPHY

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SMA is characterized by motor neuron loss and muscle atrophy due to reduced levels of survival of motor neuron (SMN) protein. RG7916 is an orally administered, centrally and peripherally distributed small molecule that modulates SMN2 pre-mRNA splicing towards the production of full-length SMN2 mRNA and increases SMN protein levels. SUNFISH (NCT02908685) study assesses the safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD) and efficacy of RG7916 in patients with Type 2 or Type 3 spinal muscular atrophy (SMA).

SUNFISH is a multicenter, double-blind, placebo-controlled trial (randomized 2:1, RG7916:placebo) in patients with Type 2 or 3 SMA aged 2–25 years. SUNFISH comprises two parts: Part 1 is evaluating the safety, tolerability and PK/PD of several RG7916 dose levels (n=51); the pivotal Part 2 is assessing the safety and efficacy of the RG7916 dose level selected from Part 1 (n=168).

We have previously presented an early analysis of SUNFISH Part 1, which showed that RG7916 administration results in a dose-dependent increase in full-length SMN2 mRNA and a concomitant decrease in SMN2Δ7 mRNA. Recent analysis of SMN protein levels in whole blood showed that in patients with SMA, SMN protein increased in a dose-dependent manner up to median 2.5-fold. The safety, tolerability and PK/PD data from Part 1 informed the selection of a RG7916 dose level for SUNFISH Part 2 predicted to lead to clinically efficacious increases in SMN protein. To date, no drug-related adverse events leading to withdrawal have been observed. We will provide a detailed SUNFISH update with novel biomarker, PK and full unblinded safety results from Part 1.

n SUNFISH Part 1, RG7916 treatment modulated SMN2 mRNA and increased SMN protein dose-dependently. The clinical benefit of the selected dose level is being assessed in SUNFISH Part 2, which is currently recruiting globally.

Study sponsored by F. Hoffmann-La Roche

NOTES

P47: AVXS-101 TRIAL EXPERIENCE: CHOP-INTEND DETECTS EARLY IMPROVEMENTS IN INFANTS WITH SMA TYPE 1 BUT IS NOT SENSITIVE TO CONTINUED ADVANCES IN MOTOR FUNCTION

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OBJECTIVE:
Evaluate the utility of the Infant Test of Neuromuscular Development (CHOP-INTEND) to quantify change in infants participating in AVXS-101 gene replacement therapy trial (NCT02122952).

BACKGROUND:
SMA1 is a neurodegenerative disease that results in motor function decline. The CHOP-INTEND was the obvious motor outcome for AVXS-101 study due to its published reliability and ability to quantify the natural decline of infants with SMA1. By 6 months, almost no children with SMA1 achieve a score >40 points (maximum 64) on the CHOP-INTEND.

DESIGN/METHODS:
Twelve patients (median age = 3.1 months) received a one-time intravenous dose of AVXS-101 at a proposed therapeutic dose in an open-label study (NCT02122952).

RESULTS:
Rapid response in CHOP-INTEND scores were observed with mean increases of 9.8 points at 1 month and 15.4 points at 3 months post-dosing; by 5.3 months of age 11/12 patients achieved a score of >40. 9/12 achieved sitting unassisted and rolling without a commiserate change in score (Jan 20, 2017). Discordant with achievement of sitting unassisted at a mean of 13.5 months post-dose, a plateau of a mean CHOP-INTEND score of 54 was observed at approximately 9.9 months post-dose in children who did not achieve the scale maximum.

CONCLUSIONS:
CHOP-INTEND is reliable at detecting early treatment impact in children participating in the AVXS-101 study, demonstrating departure from natural history. However, the upper range in the CHOP-INTEND scale appears to be insensitive to motor milestone achievement that was previously unanticipated. To accurately detect motor abilities beyond that normally achieved by SMA1 children with effective therapeutic intervention a rapid transition/co-administration of an assessment tool with advanced items is needed (ex: Bayley-III) or the CHOP-INTEND may require addition of more advanced items. We will present items that may improve the sensitivity to new motor function that was previously unanticipated.

NOTES

P48: RELATIONSHIP BETWEEN CENTRAL AND PERIPHERAL SMN PROTEIN INCREASE UPON TREATMENT WITH RO7034067 (Rg7916)

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RO7034067 (RG7916) is an oral, small-molecule SMN2 pre-mRNA splicing modifier that distributes into CNS and peripheral tissues. RO7034067 was designed to penetrate the CNS avoiding interaction with human MDR1, a transport protein that restricts brain penetration. RO7034067 is under investigation in SUNFISH, FIREFISH and JEWELFISH. We assessed the tissue distribution and the effect of RO7034067 (RG7916) on survival of motor neuron (SMN) protein expression in animal models of spinal muscular atrophy (SMA).

Distribution of RO7034067 into brain and CSF was assessed in two SMA transgenic mouse models (Δ7 and C/C-allele), rats and cynomolgus monkeys after single and repeated daily oral doses, up to 39 weeks. SMN protein levels in blood, brain and muscle of SMA transgenic mice were monitored.
Total drug levels were similar in plasma, muscle and brain of mice, rats and monkeys. CSF levels reflected those of free, non-bound compound in plasma. For example, oral dosing of RO7034067 at 3 mg/kg/day for 7 days in monkeys (n=2), total plasma concentrations (794 ng/ml) matched brain (783 ng/g) and muscle (668 ng/g) concentrations, whereas CSF drug level were within the same range of free compound concentration in plasma. This tissue distribution was maintained in monkeys receiving RO7034067 daily for 39 weeks.

SMN protein level increase in brain and muscle of SMA transgenic mice was dose-dependent. For example, 7 days' dosing in Δ7 mice (n=7) resulted in a similar increase in the SMN protein levels in brain and muscle (0.1 mg/kg/day: brain 28%, muscle 32%; 1 mg/kg/day, brain 206%, muscle 210%). SMN2 splicing modifiers with structural similarities to RO7034067 showed a parallel SMN protein increase in both brain and blood of SMA mice.

PK/PD relationships in animal models imply that SMN protein increases seen in patients' blood following RO7034067 treatment are expected to yield parallel SMN protein increase in CNS and muscle.

Study sponsored by F. Hoffmann-La Roche

NOTES

P49: A MODULAR APPROACH FOR SMA DATA MANAGEMENT: A WAY TO FACILITATE COLLABORATIVE AND LARGE RESEARCH PROJECTS

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Clinical SMA research nowadays requires large amounts of data to test important new hypotheses. Clinical database management is challenging in terms of enrolling sufficient numbers of patients, data quality control. Avoiding the existence of separate coexisting databases that cause data sets to become heterogeneous and affect the data quality over time is a clear example.

Within the domain of rare diseases, it can be even more challenging to find affordable data solutions or services that support collaborative initiatives that would allow larger research studies.
In recent years a collaborative effort within the ALS research community has led to a data infrastructure where researchers from different countries can set up or participate in new studies. This has led to great new insights in the understanding of ALS as a disease and has facilitated multicenter trials.

Its success relies on a standardized design of a core minimal dataset. New data elements can subsequently be added and linked with one another through the use of unique identifiers for cases and controls. One individual can be included in multiple 'building blocks' (i.e. projects or studies. This modular approach encourages growth and increases opportunities for integration of projects and collaboration, while data management remains relatively simple. It facilitates applying general processes for data capture, entry, quality control and management of data access on a larger scale.
We have applied this approach to create a database for our national SMA cohort study and have included standardized, core clinical data of approximately 300 SMA patients. From there additional, newly generated data was added relatively easy with the start of new research projects and collaborations, as well as the incorporation of all bio-bank data for our SMA research projects.
The modular nature of the database allows for easy further expansion of data while keeping access control simple and straight forward: it can be managed for each study as a whole or even on the level of a single variable.

Successful integration of core clinical data with all sorts of additional research data can be brought back to simple building blocks: standardized, core data and additional components. When managed with care, this approach facilitates research projects, optimizes data quality and could be used for future international collaborations.

NOTES

P50: THE USE OF NUSINERSEN IN THE "REAL WORLD": THE UK AND IRELAND EXPERIENCE WITH THE EXPANDED ACCESS PROGRAM (EAP)

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Following the promising results of the large international phase II ENDEAR clinical trial, nusinersen is the first drug receiving FDA and EMA approval and is currently offered under EAP in several European countries to patient with type 1 SMA. We report the experience in administering this drug under the EAP in 16 specialised centres in UK and Ireland. From March to October 2017, 63 patients (25 males, 38 females) were treated with nusinersen; the intrathecal injections were performed using topical anaesthetic cream in most cases, few patients older than 12 months required general anaesthetic. Patients were assessed at baseline and post loading doses (either at 4th or 5th dose) according to the schedule of injections, with at least one or a combination of the following: neurological examination, HINE-2 and CHOP-Intend. Information on SMN2 copy number, nutrition and respiratory function was collected. At baseline the patients' median age was 14 months (range 1 month to 9.5 years); SMN2 copies number was available in 51/63 patients: 38 have two copies, 12 have three copies and 1 patient has one SMN2 copy. Twenty-three patients received at least five injections. The mean CHOP-intend total score at baseline was 25/64 (range 5- 52), and 36/64 (range 9- 51) at the 5th injection. Most patients improved the CHOP-intend score (1-17 points); few remained stable, only one dropped from 52 at baseline to 46 at the 5th injection due to limited mobility secondary to a bone fracture, but scored 58 after the 4th injection. HINE-2 scores were available in 16 patients at baseline and at 5th injection; an improvement of at least 2 points was observed in 8 patients with no cases of motor regression. At baseline 33/63 patients were receiving non-invasive ventilation (NIV), 14/33 for >16 hours/day; one had tracheostomy. In 5 patients a reduction of the hours on NIV was noted; four additional patients needed to start NIV while on treatment. Four patients died while receiving nusinersen but the cause of death was considered non-related to the drug. To date patients tolerated the lumbar puncture procedure well and no side effects or adverse drug related events were noted. Our data confirm the safety of intrathecal nusinersen treatment in a heterogeneous population of SMA type 1 as well as a degree of motor function improvement._ More longitudinal data is necessary to evaluate the long-term benefits, the respiratory muscle response and the variables responsible for optimal responds.

NOTES

P51: HOW IMPORTANT IS THE EARLY TREATMENT OF SMA TYPE 1 WITH NUSINERSEN? A SPANISH SERIES OF 17 CASES

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INTRODUCTION
Nusinersen is the first treatment approved for SMA. The phase 3 trial proved to be useful in more than 50% of cases. The presymptomatic NURTURE trial (of cases supposed to be SMA 1 to SMA 3) shows better results than those of symptomatic patients.

OBJECTIVES
To review the results of treatment in a Spanish series and to study the relation of age at start of the treatment and outcome.

METHODS
17 patients were treated, 6 of them (GROUP 1) at age 7 month or before (4 participated in RCT), and 11 cases (GROUP 2) were treated starting after 7 month of age (2 in RCT phase3, but one of them with sham procedure). 16 patients had 2 copies of SMN2 and 1 had 3 copies. The onset of the disease had been similar in both groups, 2,1 months in GROUP 1 and 3,3 month in GROUP 2. Follow up of 7,9 months (range 1-29). A total of 82 intratecal injections of nusinersen. The patients were examined with the scales AIMS, HINE, part 2, and CHOP INTEND.They received in average 4.8 doses (6 and 4 respectively in GROUP 1 and GROUP 2).

RESULTS
GROUP 1, treatment at age 7 months: 2 patients died, and 4 are alive, and improved. Now they aged 23-35 months, have received after 7-10 infiltrations, and improved in the scales in average 16 points (AIMS), 7,5 (HINE) and 21 (CHOP). Two of them got and still maintain the bipedestation. Only 1 needed tacheostomy, and 2 were intubated because acute diseases but extubation was possible afterwards.
GROUP 2: 11 cases treated after 7 month of age: 2 patient died and 9 alive. Only2 of them improved in scales AIMS and HINE, and only 3 improved more than 4 points in CHOP INTEND.Average improvement of these 9 cases: 1 point (AIMS), 0,2 points (HINE) and 4.2 points (CHOP INTEND). 5 patients with tracheostomy and in 1 the tube needed because an acute pulmonary infection could be retired.

CONCLUSION
An important difference in evolution was seen in cases treated early. Before 7 month of age most cases improved, some of them remarkably. And after _this age little improvement can be expected.

NOTES

P52: CLINICAL CHALLENGES IN THE TREATMENT OF SPINAL MUSCULAR ATROPHY (SMA) WITH NUSINERSEN

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In June 2017, the European Medicines Agency approved the antisense oligonucleotide (ASO) nusinersen for the treatment of all for all 5q-associated spinal muscular atrophy (SMA) types. The 2´-O-methoxyethyl phosphorothioate modified antisense drug nusinersen has to be administered intrathecally within an initial saturation period (day 0, 14, 28 and 63) to warrant drug saturation followed by a frequent maintaining application in 4 month intervals. Due to the clinical features of SMA, the clinical application of the ASO by lumbar punction is clinically challenging considering the frequently observed scoliosis, previous orthopedic surgeries or the need of sedation especially in infants or young children.

We are currently treating 19 patients with 5q-associated SMA (type 1 to 3) in the age ranging from 8 months up to 52 years, 7 with permanent or intermittent ventilatory support. We report from clinical observations and challenges regarding the treatment procedures that often require interdisciplinary collaborations (neurologists, neuroradiologists, pediatrics, orthopedics and anesthesiologists). Here, CT-graphic supported lumbar punction is the most challenging issue regarding drug application and was necessary in 8 of the patients. In detail, 4 of these 8 patients had prior lumbar spine surgery (e.g. spondylodesis) whereas a severe scoliosis was clinically evident in the other 4 patients that lead to frustrating lumbar punction without imaging support. Further, in infants and children, topical anesthetics and opioids (e.g. nalbuphine) in moderate dosage had to be administered to warrant successful lumbar punction. Indeed, adolescents and adults received solely topical and local anesthetics for lumbar punction with the exception of long-lasting punction procedures where patients had to be treated with benzodiazepines such as midazolame, opioids or anesthetics such as s-ketamine.

However, adolescent or adult SMA-patients with less severe disease progression are currently also under treatment with nusinersen in our department but the available therapy monitoring strategies and outcome variables regarding clinical efficacy such as motor scales seem not be appropriate considering that main motor functions have been clinically developed. Thus, we suggest further clinical and laboratory features to monitor the clinical efficacy especially in older and those with less disease progression such as cerebral spinal fluid, imaging and electrophysiological parameters.

NOTES

P53: SAFETY AND EFFICACY FINDINGS IN THE FIRST-IN-HUMAN TRIAL OF THE ORAL SPLICE MODULATOR BRANAPLAM IN TYPE 1 SPINAL MUSCULAR ATROPHY (SMA): EFFECT OF AGE AT FIRST TREATMENT

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INTRODUCTION:
LMI070X2201 (NCT02268552) is an open-label, multi-part, first-in-human study of oral branaplam (formerly known as LMI070) in infants with Type 1 spinal muscular atrophy with 2 SMN copies. The purpose of Part 1 of this study was to evaluate the safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD) and efficacy after 13 weeks of treatment and to estimate the Maximum Tolerated Dose (MTD) and optimal dosing regimen of enterally administered branaplam in patients with Type 1 SMA. Treatment extensions are allowed after the initial 13 week treatment period.

METHODS:
Safety assessments and efficacy outcomes were collected for an additional 13 months following completion of the initial 13 week treatment period.

RESULTS:
14 patients enrolled in the trial. One patient failed screening. 13 patients were treated with branaplam. All continued into treatment extension period. Five patients have died during the course of the trial from disease progression as assessed by the investigators and the Data Monitoring Committee. Eight patients continue on branaplam for between 23 -30 months, with patient ages ranging from 26 to 30 months No MTD has been reached; the emerging safety profile of branaplam shows adverse events that are mostly mild, reversible and manageable. Improvements in CHOP INTEND and HINE-2 were noted in some patients. Loss of motor function was noted in several patients following lowering the dose precipitated by preclinical toxicology findings in dogs. Partial recovery of motor functions has occurred in some patients following their resumption to previously highest tolerated dose. Transition to oral administration from initial enteral tube administration has been successful. An interim analysis was conducted after a minimum of 14 months. Similar to emerging data with other modalities, younger age at time of initial dose was correlated with improved motor function.

CONCLUSIONS:
Results in this ongoing open label, first-in-human trial of branaplam in SMA Type 1 demonstrate good safety and tolerability. Improvements in CHOP INTEND and HINE-2 support continued study in SMA.

NOTES

P54: FIRST CLINICAL EXPERIENCE WITH NUSINERSEN IN SMA TYPE 2 PATIENTS - 6 MONTHS' OBSERVATION

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INTRODUCTION:
Expanded access programs (EAP) for nusinersen have been limited only to SMA type 1 patients. In France, nusinersen became early available to patients with SMA type 2 under a specific ATU procedure (autorisation temporaire d'utilisation).

METHODS:
Between April and June 2017 we initiated the treatment in 17 (12 males, 5 females) patients with SMA type 2.We performed clinical examination and physioterapist's evaluation at the beginning and after sixth months of treatment. We collected detailed medical history of infections and hospitalizations. We monitored patients for side effects of the procedure (post-lumbar punction syndrome) or the drug itself. Most procedures were realized using nitrous oxide.

RESULTS:
We report here our experience, including practical and safety issues, and our first results of clinical efficacy of nusinersen in SMA type 2. The median age of first symptoms was 11 months (6 – 16) and the median age at the start of the treatment was 3.3 years (2.2 – 10.5). 12 patients carried 3 copies of SMN2 gene, one carried 2 copies and in four cases the number of copies was not quantified. 3 patients used non-invasive ventilatory support at night and 10 patients used a cough assist. All patients were fed orally, although one patient was below 3rd percentile of weight.Up to date, the treatment was overall well tolerated. Nitrous oxide appeared to be safe and allowed the realization of the injection in good condition for the child and the practitioner. We will report the efficacy results at 6 months of follow up and the incidence of side effects.

CONCLUSION:
As in the double blind placebo controlled study, intrathecal administration of nusinersen was well tolerated. Concomitant use of nitrous oxide appeared to be a safe, well tolerated and efficient procedure. This data are to our knowledge the first "real life" report of nusinersen in SMA type 2 patients.

NOTES

P55: AVXS-101 PHASE 1 GENE REPLACEMENT THERAPY CLINICAL TRIAL IN SMA TYPE 1: CONTINUED EVENT FREE SURVIVAL AND ACHIEVEMENT OF DEVELOPMENTAL MILESTONES

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Spinal muscular atrophy (SMA) is a devastating, monogenic neurodegenerative disease. Children with SMA Type 1 (SMA1) will never sit unassisted or maintain head control. A natural history study of this patient population reported that none achieved a CHOP-INTEND score of >40 by 6 months of age (one transient exception) and 75% died or required permanent ventilation by 13.6 months. This trial explores safety and efficacy of a one-time administration of gene replacement therapy. AVXS-101 delivers the SMN gene in a one-time dose via the AAV9 viral vector, which crosses the blood-brain barrier.

In this Phase 1 trial, 15 patients with SMA1 confirmed by genetic testing (with 2xSMN2 copies) were enrolled. Patients received an intravenous dose of low dose AVXS-101 (Cohort 1, n=3) or proposed therapeutic dose (Cohort 2, n=12). The primary objective was safety and secondary objectives included survival (avoidance of death/permanent ventilation) and ability to sit unassisted (video confirmed by external independent reviewer). CHOP-INTEND scores and other motor milestones were additional objectives.

AVXS-101 appeared to have a favorable safety profile and to improve survival at data cut-off (20 January 2017). All patients reached 13.6 months free of permanent ventilation and none have died (3 live >30 months). Cohort 2 patients demonstrated improvement in motor function: 11/12 achieved head control and sat with support, and 9/12 sat unassisted. Two patients stood and walked independently.

In contrast with the natural history, a one-time intravenous administration of AVXS-101 appeared to demonstrate a positive impact on the survival of both cohorts and a dramatic, sustained impact on motor function of Cohort 2: 11/12 patients achieved CHOP-INTEND scores and motor milestones rarely or never seen in this population. A clinical update will be given at the time of presentation demonstrating continued improvements in patients.

NOTES

P56: DISEASE IMPACT ON GENERAL WELL-BEING AND THERAPEUTIC EXPECTATIONS OF EUROPEAN TYPE II AND TYPE III SPINAL MUSCULAR ATROPHY PATIENTS

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Spinal muscular atrophy (SMA) is a neurodegenerative disorder showing a broad clinical spectrum and no cure to date. To design and select evaluation criteria for the potential assessment of drugs currently being developed, the patient's perspective is critical. A survey, aiming to obtain a view on the current clinical state of European Type II and Type III SMA patients, the impact of this situation on their quality of life and their expectations regarding clinical development, was carried out by SMA-Europe member organizations in July 2015. A questionnaire was set up, translated into 8 European languages and sent out directly via electronic mailing to the targeted SMA patient population by the respective European patient organizations. We were able to collect 822 valid replies in less than two weeks. The questionnaire captured the current abilities of the respondents, their perception of the disease burden which appeared very similar across Europe despite some regional variations in care. According to the great majority of the respondents, stabilization of their current clinical state would represent a therapeutic progress for a compelling majority of the respondents to the questionnaire.lity. Improvements in CHOP INTEND and HINE-2 support continued study in SMA.

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