4th
Scientific International Congress on
Spinal Muscular Atrophy

GHENT

14th — 16th March 2024

Organised by

SMA EUROPE

Kindly hosted by

SMA | BELGIUM

ABSTRACT BOOK

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#WeAreUnique
2023 Awareness Campaign Vernissage

SMA has many different faces.

Our SMA Europe community is an example of how diverse the condition is, and how many different manners exist when it comes to living with it.

Traveler, Explorer, Journalist, Rebel… eleven individual stories which, together, resonate in one powerful poem that we want to share with you.

Join us and visit the Vernissage:
ICC Ghent, 1st floor, next to the Auditorium

Emilia Debska
Communications and Marketing Manager
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Welcome

SMA Europe takes great pride in inviting you to the 4th International Scientific Congress on Spinal Muscular Atrophy (SMA), from 14th to 16th March 2024 in Ghent, Belgium. Our Congress is the largest scientific congress dedicated to SMA and brings together scientists and young researchers as well as clinicians and other health-care professionals from all over the world.

SMA Europe is a non-profit umbrella organisation of SMA patient organisations from across Europe. We work together to create a better world for all those living with SMA. One of our core activities is to foster patient-relevant research in the field of SMA, to communicate the value generated from research, and, consequently, to ensure future support for research within our community.

The goal of our scientific congress is to bring together an international and multidisciplinary group of scientists and health-care professionals. We provide a venue to present and exchange breakthrough ideas relating to SMA, especially also in light of the patientrelevance of their findings, and to cement existing and stimulate new collaborations.

Equally, our congress is a platform for talented young researchers. Together with experienced scientists, health-care professionals, and patient experts, they will discuss, debate and dissect significant new developments and advancements related to SMA. Moreover, they will share their visions for the future of SMA research by providing an opportunity to exchange scientific evidence and clinical experiences. Therefore, we trust that the conference will lead to ever-improved treatment and care for patients living with SMA.

The International Scientific Congress on Spinal Muscular Atrophy is a unique occasion to meet face-to-face with colleagues who are as passionate about advancing the field of SMA as you are.

All together. One Goal.

SMA Europe very much looks forward to welcoming you in Ghent!

Nicole Gusset
SMA Europe CEO and President

SMA EUROPE

About us

SMA Europe is a non-profit umbrella organisation of spinal muscular atrophy (SMA) patient organisations from across Europe. We work to bring effective treatments and optimal care to everyone living with SMA.

Together, through greater understanding, we will create a better world for all those living with SMA.

All together. One goal.

Our priorities

Research

Our mission is to be active and progressive in the search for treatments for SMA. We do this through promoting and generating patient-relevant data. We are supported by a Scientific Advisory Board (SAB), composed of neuroscientists and neurologists with particular expertise in SMA.

Through our research programme, we:

- Seek to set patient-relevant research priorities
- Promote these patient-relevant research priorities in our Call for Research Proposals and our scientific congresses
- Systematically research and assess the needs and wants of people living with SMA
- Identify data gaps that are relevant to patients and fill those by:
    - producing our own patient-relevant research projects and publishing the outcome in peer-reviewed journal
    - stimulating, (collaboratively) supporting and funding research that addresses these gaps
    - facilitating communication between stakeholders in this field.

We also build our members' capacity to understand the relevance and processes of research, to allow them to become partners in funding research and in meaningfully contributing to discussions and solutions. In so doing, we make sure SMA research delivers on patients' unmet needs from a clinical, care and quality of life perspective.

We do this because we believe that developing a treatment that can truly help improve the lives of people living with SMA should be rooted in a firm understanding of the challenges those people face in their daily lives, their needs and the trade-offs they are willing to make to gain relief.

To ensure the creation of valuable treatments, all aspects of the health care system, including research prioritisation, product development, trial design, regulatory approval, access, reimbursement and treatment decisions, will need to align with their needs.

Therapy and Care

SMA Europe strives to accelerate progress in the diagnosis, treatment and care of people with SMA.

To this aim, we engage in dialogue with all relevant stakeholders, to ensure the needs and wants of people living with SMA across Europe are taken into account during the entire drug development process.

To justify a seat at all relevant tables and to be able to provide meaningful, qualified and evidence-based input, SMA Europe continuously educates and prepares individual patient advocates in key knowledge areas. In parallel, SMA Europe strives to collaborate with all key stakeholders as a respected partner, especially in the areas of drug development and regulatory affairs.

Healthcare Systems, Policy and Access

Access to diagnosis, treatment and care is fragmented in Europe. SMA Europe strives for unrestricted access to optimal available medicine, treatment, care and diagnostics, regardless of location, age, mobility or SMA type. This is the only outcome which will end the access inequalities that SMA families continue to live with today.

We address this issue by mapping and centralising information around access throughout Europe. We identify data gaps that influence access and we promote research to fill them. We support our members by sharing knowledge and coaching them to advocate efficiently in their own country. At a European level, we partner with and influence all stakeholders in relevant areas of healthcare and research, wherever impact can be made, bearing in mind that responsibilities in access tend to fall at national level and are limited at European level.

SMA EUROPE
Member organisations

Belgium
SMA Belgium
www.spierziektenvlaanderen.be
www.telethon.be

SMA Belgium is an umbrella organisation created by the SMA working groups of Spierziekten Vlaanderen (Flemish neuromuscular organisation) and ABMM (Walloon neuromuscular organisation).

SMA Belgium is dedicated to building communication networks between families with SMA about progress in scientific research, available treatment and public assistance.
Finally, but not least, to strengthen the patient's voice in the drug development process, to collaborate with various stakeholders to optimise the drug-development path from the laboratory to the patient.

Cyprus
MDA Cyprus
www.mdacyprus.org

The Cyprus Muscular Dystrophy Association (MDA Cyprus), was founded back in 1986 and its members are children and adults from all over Cyprus. MDA Cyprus consists of patients who suffer from neuromuscular diseases such as Duchenne Muscular Dystrophy, Myasthenia Gravis, Amyotrophic Lateral Sclerosis (ALS), Peripheral Nerve Diseases like Charcot-Marie-Tooth Disease, Spinal Muscular Atrophy and more than 50 other types of Muscular Dystrophy.

Czech Republic
SMÁci
www.smaci.cz

The Czech SMA Patient Organisation – SMÁci, supports patients and their families with their SMA quest, striving to establish a communication channel between all parties involved and helping to achieve a smooth execution of all available steps to improve quality of life. Since its creation in 2016, SMÁci has had a key role in advocating for access to treatment for people with SMA in Czech Republic.

Denmark
Muskelsvindfonden
www.muskelsvindfonden.dk

The Danish Muscular Dystrophy Foundation unites people and relatives with all types of neuro muscular diseases. The organisation consists of four units: a member based association; an event and fundraising organisation which organises a music festival and a circus-theatre; a highly specialised centre for neuromuscular diseases; and the Musholm holiday, sports and conference centre, internationally awarded for its outstanding accessibility and run as a social enterprise. Together, these four units work on advocacy, awareness, research, and empowerment to improve treatments and create opportunities for living a good life with a neuro muscular disease.

Finland
SMA Finland
www.smafinland.fi

SMA Finland was founded in 2018 by group of active SMA patients and parents with children who have SMA. The primary goal of the association is to provide access to medical treatment to all SMA patients, despite the type or age.
Other important goals for SMA Finland are also to improve the quality of life of individuals with SMA and to create awareness about SMA with authorities, healthcare professionals and the general public.

France
AFM-Téléthon
www.afm-telethon.fr

The French Muscular Dystrophy Association (AFM) federates patients with neuromuscular diseases and their parents. Thanks in great part to donations from France's annual Telethon, the AFM-Telethon has become a major player in biomedical research for rare diseases in France and worldwide. It currently funds about 37 clinical trials in different genetic diseases affecting the eye, blood, brain, immune system, and muscles... Thanks to its Genethon research lab, the AFM-Telethon stands out through its unique ability to produce and test its own gene-based medicines.

Germany
DGM
www.dgm.org

The German Society for people with Muscular Diseases (DGM), founded in 1965 at the initiative of parents, is the oldest and largest patient organisation for people with muscular diseases in Germany. Its main objective is to the advance research in the field of muscle disorders and treatments.

DGM provides advice, assistance, and the opportunity to exchange experiences with other stakeholders, also being committed to the concerns of the other stakeholders within health policy.

Greece
MDA
www.mdahellas.gr

MDA (**Muscular Dystrophy Association Hellas**) focuses on improving the lives of individuals with neuromuscular disorders by working with government agencies, universities and various institutions and individuals to further research on neuromuscular disorders and to promote early diagnosis. The organisation runs events and education programmes for patients focused on advocacy, clinical trials, novel medicines, and technologies while also supporting and educating doctors. MDA Hellas has established and supports 3 Specialised Neuromuscular Units in Greece.

Hungary
SMA Hungary
www.smahun.hu

The primary goal of the Foundation is not to directly support patients, but to support domestic treatment (e.g., additional personal resources), to exchange information about treatment options and to organise and finance events related to this (in particular study trips abroad, lectures of foreign doctors in Hungary).

Iceland
FSMA Iceland
www.fsma.is

FSMA Iceland was formally established in 2002 and is an association of families and individuals who suffer from Spinal Muscular Atrophy (SMA). Its purpose is to protect the interests of the persons with SMA and their families and to contribute to finding a cure. FSMA does this by fundraising to support research into the science of the disease; providing information to members of FSMA in Iceland on the progress of research on the disease, as well as other useful information related to the disease; to hold meeting on issues SMA; to disseminate information in the media and to the media about issues pertaining to SMA; to contribute to the transportation of individuals with SMA and their families.

Ireland
SMA Ireland
www.smaireland.com

The Spinal Muscular Atrophy Ireland Foundation is the collective voice for the adults and children with SMA in Ireland. The organisation supports people and families by: providing information and a network of contacts; raising awareness of SMA and campaign for screening; lobbying Government and the Health Service for access to treatment; liaising between pharmaceutical companies and patients/families; facilitating ongoing research by coordinating patient volunteers; and maintaining connections with SMA organisations in other jurisdictions.

Italy
Famiglie SMA
www.famigliesma.org

The Association of Families of SMA is a non-profit NGO founded in 2001 by a group of parents of children with SMA. The Association is a point of reference for medical and scientific researchers and for all the families of children with SMA. It aims to inform families about progress in scientific research, available treatments and assistance to which they are entitled to from public institutions; to promote and support scientific research in SMA and possible therapies; to communicate developments in clinical trials of drugs and therapies as well as the participation of Italian research organisations in clinical trials for SMA abroad, also solving legal problems, bureaucratic and organisational related to such participation.

Macedonia
Stop SMA
www.stopsma.mk

The Association of persons with Spinal Muscular Atrophy STOP SMA is an association of citizens, established for the purpose of realising, protecting, and promoting the rights and interests of the persons with SMA and their custodians. This includes their rights and interests regarding health protection as well as their rights to treatment, social protection, education, and employment.

The Netherlands
Prinses Beatrix Spierfonds
www.prinsesbeatrixspierfonds.nl

The Prinses Beatrix Spierfonds, a foundation for over 200.000 people with a neuromuscular disease in the Netherlands, aims to eliminate all neuromuscular diseases by means of scientific research. The organisation finances and stimulates research

aimed at developing therapies for neuromuscular diseases. Because this can be a long and strenuous process, the organisation simultaneously supports research in improving quality of life.

The Netherlands
VSN - Spierziekten Nederland
www.spierziekten.nl

Spierziekten is an association of and for people with a neuromuscular disease. Its activities consist of providing information, organising mutual support, and stimulating scientific research, including international cooperation in the fields of research and of the development of therapies.
Spierziekten runs a series of initiatives focused on the improvement of social and medical care for people with neuromuscular diseases and organises various information and dissemination activities each year.

The association collaborates closely with relevant experts to improve diagnostic procedures, care, rehabilitation, and genetic counselling while also maintaining a network of regional groups and national diagnosis-bound support groups.

Poland
Fundacja SMA
www.fsma.pl

The Polish SMA foundation, formed by parents of children with SMA, has the following goals: to conduct activities for people with SMA and their loved ones, aimed at combating exclusion, increasing independence and improving their quality of life; to increase the awareness of SMA, by disseminating knowledge in genetics, diagnostics, standards of care and treatment methods; to increase the availability of methods and techniques for diagnostic, therapeutic, rehabilitative and related products and technology solutions; to support system solutions, particularly in health care and social security, taking into account the needs of people with SMA and their loved ones.

Portugal
APN
www.apn.pt

APN (**Associação Portuguesa de Neuromusculares**) focuses on creating and promoting better quality of life for people living with neuromuscular diseases. Its members are people with muscular diseases, family members, doctors, and other health professionals. APN's actions include advocacy, direct support to people with neuromuscular diseases and their members and support to medical research.

Romania
Asociatia SMACARE
www.amiotrofie-spinala.ro

SMACARE Association is a non-governmental and non-profit organisation that aims to protect the rights and interests of people affected by spinal muscular atrophy and to improve their lives. The association was founded at the initiative of some parents whose daughter is affected by Spinal Muscular Atrophy Type II. Helped by relatives and friends and encouraged by doctors decided to start on this road to change the mentality of Romanians about people with disabilities, to make this disease known and to build a communication network between people with muscular spinal atrophy, their families and doctors.
Asociatia SMACARE aims to:

- Improve the quality of live of individuals with Spinal Muscular Atrophy;
- Raise awareness about Spinal Muscular Atrophy with the general public, health-care providers, governmental organisations;
- Build a community for Spinal Muscular Atrophy families and individuals;
- Funding for Spinal Muscular Atrophy projects;

Russia
SMA Family Foundation Russia
www.f-sma.ru

The SMA Family Foundation Russia, established by parents of children with SMA, supports and empowers families with people living with SMA. The Foundation focuses on advocating for better services, raising public awareness and funding family's special needs that are not reimbursed by national healthcare service. Its main goals are: to build strong basis for the improvement of the quality of life of individuals with SMA and their families; to fulfil the strong need of information and best care practices; and to promote the development of medical and non-medical care for SMA.

Serbia
SMA Serbia
www.smasrbija.rs/en/support

SMA Serbia, established by parents of children with SMA and adults living with SMA, aims at increasing the quality of life of those living with SMA and their families. The association strives to raise public awareness, to protect the interests and the rights of people with SMA and their families and to improve their social care and medical support. The aim is to build a community for SMA families and individuals, to advise them and to make their lives easier. SMA Serbia's final intention is to get the right treatment for every single patient, children and adults, so that they grow, improve and prosper in many fields in life.

Spain
FundAME
www.fundame.net

Spinal Muscular Atrophy Foundation (FUNDAME) is a non-profit, private foundation, established in 2005 and made up of patients affected by SMA and their relatives. FUNDAME strives to find ways to improve the quality of life of those affected by SMA and to promote research into this disease.
FUNDAME supports research at both national and international level, in order to bridge the gap between today and the day a cure for SMA is available. In the meantime, FundAME seeks ways to improve the quality of life of those affected by the disease.

Sweden
NSMA
www.nsma.nu

NSMA (Nätverket för spinal muskelatrofi) is a patient association which aims to bring together people who work and live with SMA in order to exchange, assist and inform; to be the natural platform for exchange and support for people living or working with SMA in Sweden; to raise awareness about SMA in Sweden; to influence public opinion and policy makers in different social and health care organisations on issues related to SMA; and to establish a Swedish care program for SMA with information and guidelines for different treatments.

Switzerland
SMA Schweiz
www.sma-schweiz.ch

SMA Schweiz operates to develop and optimise therapies for people with SMA. The goals of SMA Schweiz are to strengthen the patients' voice in the drug development processes, to collaborate with various stakeholders to optimise the drug-development path from the laboratory to the patient, and to educate people affected by SMA and their families, as well as the general public.

Turkey
SMA Benimle Yürü
www.smabenimleyuru.org.tr
www.sma.org.tr

In Turkey, the SMA Community, is being represented by delegates from two organisations: SMA Benimle Yürü and Turkey SMA Foundation.

Ukraine
CSMA
www.csma.org.ua

CSMA (Children with SMA) unites both parents of children and people with SMA to foster exchanges of ideas to solve, at least partially, existing problems. CSMA also supports the maintenance of a national registry of patients with SMA and provides information on SMA care related topics.

United Kingdom
SMA UK
www.smauk.org.uk

SMA UK was established by a mother whose baby died aged 7 months from SMA. She set up the charity under the name of 'Jennifer Trust for Spinal Muscular Atrophy', now SMA UK, to offer support and hope to other families affected by the different types of SMA. SMA UK advocates for better services and access to new treatments so that people affected by SMA are supported, empowered and enabled to live full lives. The charity is also committed to help fund and facilitate research and to raise public and professional awareness of SMA.

PROGRAMME

Ghent, Belgium

13 March 2024

SMAdvocacy Event

The Global SMAdvocacy Event brings together patient advocates from all over the globe to engage with current issues in SMA patient advocacy. Participants share advocacy experiences and learn from each other while elaborating strategies towards concrete advocacy goals.

Discussions will focus on challenges like equity in access to SMA therapies and standards of care, reimbursement for SMA treatments and new-born screening, but also on how to make the best of national registries or influence policy.

ECR Event

SMA Europe is organising an international event geared towards fostering the relationship between early career researchers (ECRs) and SMA patient advocates. This will be a unique opportunity for ECRs to engage with the broader SMA community and to bridge the gap between their laboratory work and the lived experiences of the condition they are working on.

SMA EUROPE

WEDNESDAY 13th MARCH 2024 • Pre-Congress Day

	Global SMAdvocacy Event	BAEKELAND ROOMS 1-3
09:00 - 12:30	Morning activities	
10:30 - 11:00	**COFFEE BREAK**	
11.00 - 12:30	Morning activities	
12:30 - 13:30	**LUNCH**	
13:30 - 14:30	**Vernissage of SMA Awareness Campaign 2023**	
14:30 - 16:00	Afternoon activities	
16.00 - 16.30	**COFFEE BREAK**	
16.30 - 18.30	Afternoon activities	
18.30 - 21.00	**DINNER**	

	ECR Event	GUISLAIN 1
13:30 - 14:30	**Vernissage of SMA Awareness Campaign 2023**	
14:30 - 16:00	Afternoon activities	
16:00 - 16:30	**COFFEE BREAK**	
16.30 - 18.00	Afternoon activities	
18.30 - 21.00	**DINNER**	

THURSDAY 14th MARCH 2024

08:30 - 09:30 **REGISTRATION & WELCOME COFFEE IN THE EXHIBITION AREA**

09:30 - 09:45 **Welcome SMA Europe** — AUDITORIUM
Nicole Gusset - SMA Europe CEO and President

09:45 - 10:00 **Welcome to Belgium**
Hilde Crevits, Deputy Minister-President of the Government of Flanders, Flemish Minister for Welfare, Public Health and Family

Karel Everaert, Head of Clinics Functional Urology, UZ Ghent

10:00 - 10.45 **Is SMA cured? The future of SMA research**
Panel discussion with Yasemin Erbas, Cécile Martinat and Giovanni Baranello
Moderator: Ankita Batla

10.45 - 10:55 **Opening of scientific & clinical programme**
Thomas Gillingwater, Chair of Scientific Committee

11.00 - 11:30 **COFFEE BREAK AT THE EXHIBITION AREA**

11:30 - 13:00 AUDITORIUM
Session 1 - Rehabilitation in SMA - Motor neuron and spinal cord parthology
Chair: Farnaz Nickpour, University of Liverpool, UK

O1 **Facilitating neuromuscular junction recovery after treatment in mouse models of spinal muscular atrophy**
Inga Partlova, University of Edinburgh, UK

O2 **Respiratory muscle training in patients with spinal muscular atrophy - Results of a randomized controlled trial**
Kim Kant-Smits, Wilhelmina Children's Hospital, University Medical Center Utrecht,The Netherlands

O3 **Bone health in adult spinal muscular atrophy (SMA) patients: Is it a forgotten risk?**
Channa Hewamadduma, University of Sheffield, UK

O4 **Scoliosis progression in spinal muscular atrophy type II: A comparative study between treated and untreated patients**
Giorgia Coratti, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy

13:00 - 14:15 **LUNCH BREAK AT THE EXHIBITION AREA**

13.10 - 14.10 **Industry Sponsored Session** — T. VAN RYSSELBERGHE

THURSDAY 14th MARCH 2024

14:15 - 14:30 **Flash poster presentations, Session 1** — AUDITORIUM

P141 **Pain in spinal muscular atrophy patients**
Kursat Bora Carman, Eskisehir Osmangazi University, Turkey

P72 **Criteria for identification and accurate quantification of spinal motor neurons in healthy and disease mouse models**
Leonie Sowoidnich, Carl-Ludwig-Institute of Physiology, Germany

P13 **Longitudinal changes in compound muscle action potential (CMAP) and their association with motor function in children with infantile-onset SMA in ENDEAR/SHINE**
Michelle Farrar, UNSW Sydney, Sydney, Australia

P50 **Dopaminergic system role in a C. elegans model of SMA**
Giada Onorato, Institute of Biosciences and BioResources (IBBR), CNR, Italy

P121 **A Combined Examination of Novel Rapid Bedside Plasma-SMN Analysis and Muscle Ultrasound May Help to Early Screen & Monitor Children with Spinal Muscular Atrophy in Clinical Setting**
Dian K. Nurputra, Universitas Gadjah Mada, Indonesia

P12 **FUS protein expression and distribution in the myopathology of 5q-associated spinal muscular atrophy type 3**
Heike Kölbel, Universitätsmedizin Essen Germany

14:30 - 16:00 **Session 2 - Optimising gene-directed therapeutics** — AUDITORIUM
Chair: Rafael Yáñes Muñoz, Royal Holloway, University of London, UK

O5 **RNA perturbation in Cerebrospinal Fluid of Spinal Muscular Atrophy Patients treated by Nusinersen**
Stella Gagliardi, IRCCS Mondino Foundation, Pavia, Italy

O6 **Direct evidence of deletion and gene conversion as origin of SMA alleles revealed by long-read sequencing**
Maria Zwartkruis, University Medical Center Utrecht, The Netherlands

O7 **Characterising the effect of AAV9 mediated gene therapy on the epigenetic and transcriptional stability of SMA mice**
Piera Smeriglio, Institut de Myologie, Paris, France

O8 **Enhancing In Vitro SMN Protein Expression and Cell Viability through Xeno-Nucleic Acid-Based ASOs in Spinal Muscular Atrophy**
Cihan Tastan, Uskudar University, Istanbul, Turkey

16.00 - 16.30 **COFFEE BREAK IN THE EXHIBITION AREA**

16.00 - 17.30 **Poster session 1** — MINNEPLEIN

17:30 - 18:30 **Parallel workshops**
Towards a Research Agenda for SMA — T. VAN RYSSELBERGHE
Tom Gillingwater and Nicole Gusset

Stem cell models — H. VAN EYCK
Cécile Martinat

18:45 **WELCOME RECEPTION IN THE EXHIBITION AREA**

FRIDAY 15th MARCH 2024

08:15 - 09:30 **REGISTRATION & WELCOME COFFEE IN THE EXHIBITION AREA**

08:20 - 09:20 **Industry Sponsored Session** **T. VAN RYSSELBERGHE**

09:30 - 11:00 **Session 3 - SMN beyond the motor neuron** **AUDITORIUM**
Chair: Melissa Bowerman, university of Keele, UK

O9 **Selective loss of excitation and p53 activation cause cerebellar circuit pathology in spinal muscular atrophy**
Sandra Wittig, Carl Ludwig Institute for Physiology, Leipzig, Germany

O10 **Microvascular dysregulation: The missing link in SMA pathogenesis?**
Hazel Allardyce, University of Aberdeen, UK

O11 **Translational defects in multiple tissues from the Smn2B/- Mouse model of SMA**
Gaurav Sharma, Institute of Biophysics, CNR, Trento, Italy

O12 **Identification of autonomic cardiovascular dysfunction in adult SMA patients: Towards the understanding of multisystem involvement in SMA**
Silvia Bonanno, Fond. IRCCS Ist. Neurologico C. Besta, Milan, Italy

11.00 - 11:30 **COFFEE BREAK IN THE EXHIBITION AREA**

11.30 - 13:00 **Session 4 - Developmental aspects of SMA** **AUDITORIUM**
Chair: Giovanni Baranello, University College London, UK

O13 **Time-course characterization of cortical projection neuron's alterations and development in delta7 SMA mice**
Roberta Schellino, Dept. of Neuroscience and Neuroscience Institute Cavalieri Ottolenghi, University of Turin, Italy

O14 **Transcriptional Mechanisms Differentiating Resilient and Vulnerable Spinal and Cranial Motor Neurons from human pluripotent stem cells in SMA**
Morgan Gazzola, I-Stem, Corbeil-Essones, France

O15 **Isogenic patient-derived organoids reveal early neurodevelopmental defects in spinal muscular atrophy initiation**
Natalia Rodriguez-Muela, German Center for Neurodegenerative Diseases e.V. (DZNE), Dresden, Germany

O16 **Is SMA a developmental disease?**
Federica Genovese, University of Edinburgh, UK

13:00 - 14:15 **LUNCH BREAK IN THE EXHIBITION AREA**

FRIDAY 15th MARCH 2024

13:10 - 14:10 **Industry Sponsored Session** **T. VAN RYSSELBERHE**

14.15 - 14.30 **Flash poster session, Session 2** **AUDITORIUM**

P118 **Oro facial strength in symptomatic type 1 SMA patients treated with nusinersen: Results from a prospective study involving 4 centres Milano - Rome - Brussels - Ghent**
Charlotte Colot, Hôpital Universitaire des enfants reine Fabiola, Belgium

P17 **Exon7 Targeted CRISPR-Prime Editing Approaches for SMN2 Gene Editing in Spinal Muscular Atrophy**
Cihan Tastan, Uskudar University, Turkiye

P135 **Towards the identification of biomarkers of disease progression and response to treatment in spinal muscular atrophy**
Claudia Malacarne, IRCCS Fondazione Istituto Neurologico Carlo Besta, Italy

P64 **Unravelling the role of GABA signalling and metabolism (dys)regulation in Spinal Muscular Atrophy: results from SMAΔ7 mice cortex**
Giovanna Menduti, Neuroscience Institute Cavalieri Ottolenghi, University of Turin, Italy

P100 **Electrophysiological assessment of motor unit patterns of the median nerve in adolescents and adults with spinal muscular atrophy (SMA**
Leandra Ros, University Medical Center Utrecht, The Netherlands

P47 **Network biology-based analysis of SMA: Identification of disease relevant protein targets and altered signaling in severe and mild SMA mice**
Ines Tapken, Smatheria gGmbH - Non-Profit Biomedical Research Institute, Germany

14.30 - 16.00 **Session 5 - New avenues in SMA research** **AUDITORIUM**
Chair: Séverine Boilée, Université de la Sorbonne, Paris, France

O17 **Molecular and morphological characterization of primary patient-derived fibroblasts to study gene-targeting therapies for spinal muscular atrophy (SMA)**
Ilaria Signoria, UMC Utrecht, The Netherlands

O18 **The Spinal Muscular Atrophy gene product regulates actin dynamics**
Tobias Schüning, SMATHERIA gGmbH, Hannover, Germany

O19 **Autophagy in spinal muscular atrophy: Detrimental or a therapeutic avenue?**
Saman Rashid, University of Hertfordshire, London, UK

O20 **RAINBOWFISH: Primary efficacy and safety data in risdiplam-treated infants with presymptomatic SMA**
Michelle Farrar, UNSW Sydney, Randwick, Australia

16.00 - 16.30 **COFFEE BREAK IN THE EXHIBITION AREA**

FRIDAY 15th MARCH 2024

16.00 - 17.30 **Poster Session 2** — MINNEPLEIN

17:30 - 18:30 **Parallel workshops**

Insights from other diseases — H. VAN EYCK
Richard Finkel & Stefania Corti

Newborn screening — T. VAN RYSSELBERGHE
Eduardo Tizzano

19:30 **GALA DINNER AT D'OUDE VISMIJN**

SATURDAY 16th MARCH 2024

09:30 - 11:00 **Session 6 - Muscles in SMA** — AUDITORIUM
Chair: Frédéric Relaix, UPEC - Université de Paris Est-Créteil, France

O21 **Effect of apitegromab on motor function and patient-reported outcomes at 36 months in patients aged 2-21 years with spinal muscular atrophy**
Scott Baver, Scholar Rock, Cambridge, United States

O22 **RNA-sequencing of Type II SMA paravertebral muscle after treatment reveals two distinct molecular subtypes**
Fiorella Grandi, Centre de Researche en Myologie, Paris, France

O23 **ClC-1 inhibitor compound improves neuromuscular transmission and enhances skeletal muscle function in pre-clinical animal models of neuromuscular dysfunction**
Martin Skov, NMD Pharma A/S, Aarhus N, Denmark

O24 **Taldefgrobep Alfa: Preclinical and Clinical Data Supporting the Phase 3 RESILIENT Study in Spinal Muscular Atrophy**
Cliff Bechtold, Biohaven, New Haven, United States

11.00 - 11:30 **COFFEE BREAK AT THE EXHIBITION AREA**

11.30 - 13:00 **Session 7 - Monitoring disease outcome** — AUDITORIUM
Chair: Michelle Farrar, UNSW Sydney, Randwick, Australia

O25 **Aconitase as a marker of early pathological state in SMA: Data from spinal cord and fibroblasts**
Serena Stanga, Università degli studi di Torino, Italy

O26 **Cold-induced weakness in adolescents and adults with spinal muscular atrophy (SMA)**
Leandra Ros, University Medical Center Utrecht, The Netherlands

O27 **H-reflex as a sensitive biomarker of sensory-motor circuit dysfunction in SMA mice and patients**
Christian Simon, University Leipzig, Germany

O28 **Mass-Spectrometry-based proteomics on cerebrospinal fluid identified novel potential biomarkers for Spinal Muscular Atrophy**
Chiara Panicucci, IRCCS Istituto Giannina Gaslini, Genova, Italy

13:00 - 14:00 **GRAB A LUNCH BOX AT THE EXHIBITION AREA**

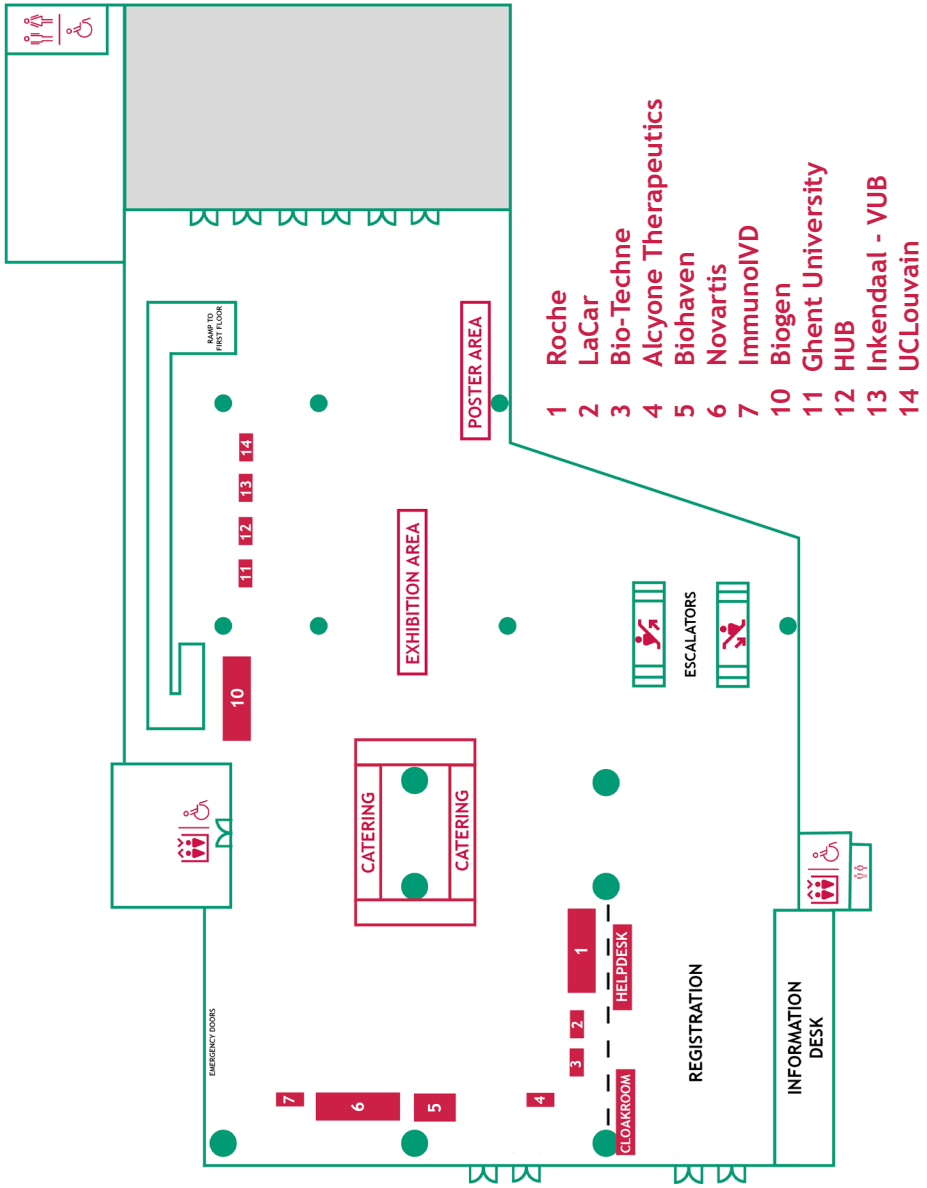
Award Ceremony and closing talk — AUDITORIUM
Chair: Tom Gillingwater

Award ceremony: Best oral presentation, best three posters
Nicole Gusset and Tom Gillingwater

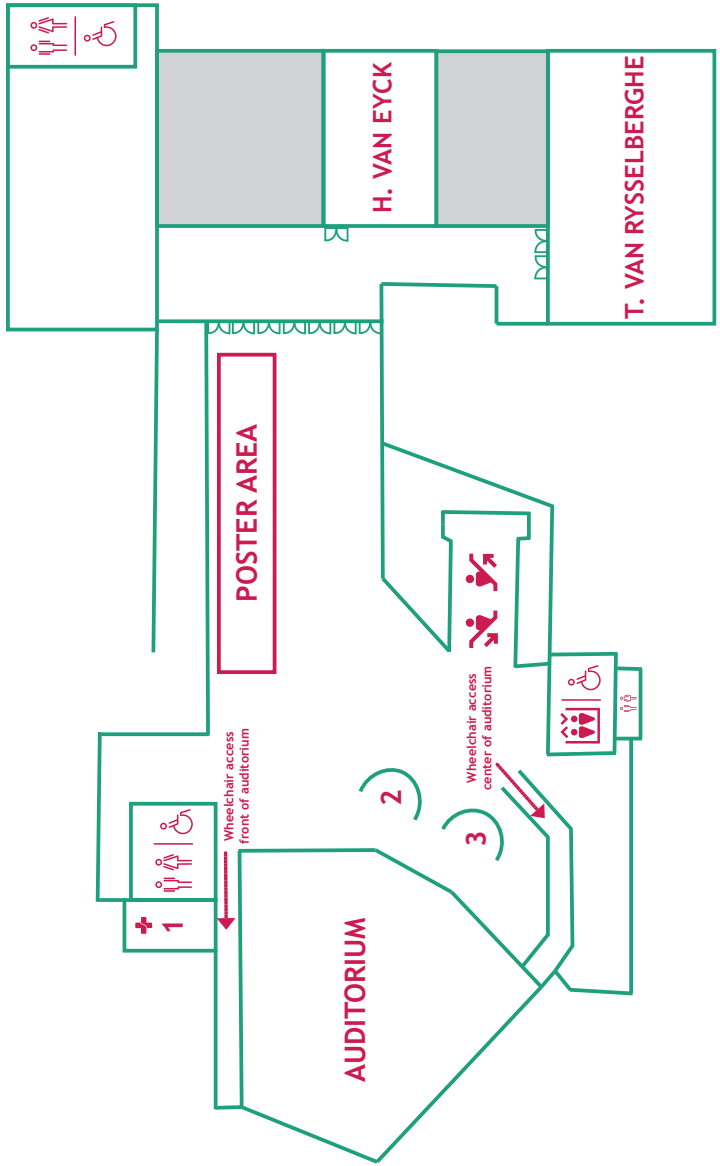
Wrap-up and highlights of the meeting - Where do we go next?
Nicole Gusset

CLOSE

Floorplan
Ground floor



Floorplan
First floor



Scientific Committee

Chair: Thomas Gillingwater
Professor of Anatomy at the University of Edinburgh and Editor in Chief of the Journal of Anatomy

Vice-Chair: Stefania Corti
Neurologist, Associate Professor of Neurology and Principal Investigator of Neural Stem Cell Lab at the University of Milan in Italy

Local host: Liesbeth De Waele
Paediatric Neurologist and Assistant Professor at the Faculty of Medicine, UZ Leuven NMRC for Children, Belgium

Nicolas Deconinck
Head of paediatric neurology department HUDERF, Head of the research unit of UZ Ghent NMRC

Melissa Bowerman
Senior Lecturer in Neuromuscular & Skeletal Disorders, School of Medicine, Keele University, UK

Claudio Bruno
Head, Center of Translational and Experimental Myology, IRCCS Istituto Giannina Gaslini, Genova, and Contract Professor in Paediatrics at the School of Medicine, University of Genova, Italy

Peter Claus
Scientific Director, CEO and Co-Founder of SMATHERIA, a non-profit biomedical research institute dedicated to spinal muscular atrophy (SMA) and other neuromuscular diseases preclinical and translational research, Hannover, Germany

Richard Finkel
Paediatric Neurologist and Director of the Center for Experimental Neurotherapeutics at St. Jude Children's Research Hospital in Memphis, Tennessee, USA

Ewout Groen
Senior Researcher, SMA Center of Expertise, UMC Utrecht Brain Center, Department of Neurology and Neurosurgery, University Medical Center Utrecht, The Netherlands

Ulrika Kreicberg
Ulrika Kreicbergs, Professor of Palliative Care for Children and Young People at the Great Ormond Street Institute of Child Health, University College London, UK

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

Christian Simon
Group Leader, Carl Ludwig Institute for Physiology, Leipzig University, Germany

Charlotte Sumner
Professor of neurology at Johns Hopkins School of Medicine, USA

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

Young Speakers

SESSION 1: Rehabilitation in SMA - motor neuron and spinal cord pathology

Kim Kant-Smits (O2)

I am Kim Kant-Smits, a pediatric physical therapist and Ph.D. student at the Wilhelmina Children's Hospital in Utrecht, the Netherlands. I finished my Bachelor of Physiotherapy in 2006 and my Master of Pediatric Physical Therapy in 2011. From 2006 to 2020, I worked as a pediatric physical therapist in primary healthcare. In 2020, I completed my Master of Clinical Health Science and started working at the Wilhelmina Children's' Hospital. My areas of expertise in care are pulmonary problems and the ICU. My research is focused on the respiratory muscles of patients with SMA. I hope to finish my thesis at the end of this year. Progressive respiratory muscle weakness is an important cause of morbidity and mortality in patients with SMA. It is important to know what treatment strategies can slow down or even stop the decline in respiratory muscle strength and lung function. Respiratory muscle training seems a promising treatment strategy for patients with SMA and respiratory muscle weakness.

Giorgia Coratti (O4)

Dr. Coratti has a B.Sc. in Neuro-Psychomotor Therapy of Developmental Age from the Catholic University of Sacred Heart (Rome), an M.Sc. in Cognitive sciences and decision making from the Università degli Studi di Milano (Milan) and a PhD in Neuroscience from the Catholic University of Sacred Heart (Rome). She works in the programme for Neuro-Psychomotor Therapy of Developmental Age for the Fondazione Universitaria Policlinico Agostino Gemelli IRCCS, Catholic University of Sacred Heart (Rome) where she assesses patients' rehabilitative needs, developing patient plans and treatment goals. Her professional interests lie in neuromuscular disorders, clinical research, paediatric neurology and psychiatry. Dr. Coratti has authored, or co-authored, 60+ peer-reviewed scientific publications and her research findings have been presented before national and international conferences.

SESSION 2: Optimising Gene-directed Therapeutics

Maria Zwartkruis (O6)

In 2017, Maria received her Bachelor's degree in Biomedical Sciences at Utrecht University. The same year, she continued with a Master's degree in Neuroscience and Cognition at Utrecht University. As part of this programme, she investigated liquid-liquid phase separation of the FUS protein in amyotrophic lateral sclerosis (ALS) under supervision of prof. dr. Ludo Van Den Bosch at VIB-KU Leuven. This first sparked her interest in neuromuscular disease. She finished her Master's degree with a literature thesis about the link between autophagy and axonal transport in neurodegenerative disease, for which she received the Talma Eykman thesis award. Having followed several courses about bioinformatics, Maria decided to move towards the genetics field. In 2020, she started her PhD on spinal muscular atrophy (SMA) genetics in the lab of dr. Ewout Groen, as part of the multidisciplinary SMA research group of prof. dr. Ludo van der Pol. With a specific interest in long-read sequencing techniques such as Nanopore sequencing and PacBio HiFi sequencing, she aims to further our understanding of complex SMA genetics, to enable more accurate prognosis and better informed treatment decisions for SMA patients.

SESSION 3: Optimising Gene-directed Therapeutics

Sandra Wittig (O9)

Sandra Wittig started her medical studies at Leipzig university in 2018 and receives a scholarship from the German Academic Scholarship Foundation. During her pre-clinical training, she found her fascination for the nervous system and neurodegenerative diseases. She therefore became member of the Simon research group at the Carl Ludwig Institute for Physiology in Leipzig as a medical thesis candidate. Her work focuses on the cerebellar pathology in Spinal Muscular Atrophy, especially the mechanisms of Purkinje cell death and synaptic impairment, aiming to decipher cerebellar contribution to motor function in SMA patients.

Hazel Allardyce (O10)

Hazel Allardyce obtained an undergraduate MSci in Human Anatomy at the University of Glasgow, Scotland, graduating in 2019. During the MSci she spent time at the Institute of Biotechnology, University of Helsinki, Finland, and at the Institute of Medical Sciences, University of Aberdeen, Scotland, in the laboratory of Professor Simon Parson. Here her research project focussed on renal pathology in a severe SMA mouse model, which marked the beginning of her career in SMA research. Hazel was then accepted for a PhD studentship in the Parson laboratory characterising treatment-naïve, post-mortem spinal cord of severe Type I SMA patients, graduating in 2023.

Hazel now works as a postdoctoral research fellow within the Parson laboratory where she continues her research on SMA patient post-mortem tissue using morphological and microscopical techniques, as well as investigating patient fluid biomarkers and SMA cellular modelling. Hazels current interests are focused on vascular dysfunction in SMA and its importance in disease pathogenesis and progression. By understanding the cause and effect of vascular abnormalities in SMA, we will obtain a more thorough understanding of the disease. This will lead feed into the design of next generation and follow-on therapies for those affected by SMA.

Gaurav Sharma (O11)

I studied Biotechnology at the National Institute of Technology, India and an M.Sc. in Molecular Biology at Katholieke Universiteit Leuven, Belgium. In May 2021, I started working on SMA as a Marie-Curie fellow and PhD student in the Viero group (Dr. Gabriella Viero) as part of the European project SMABEYOND. I am dedicated to unraveling the intricate molecular mechanisms underlying SMA. My primary interest lies in investigating translation-based biomarkers and the role of translation in SMA progression. My work is focused on studying the translation defects resulting from SMN deficiency in the SMA mouse models. By identifying potential translation-based biomarkers, my work aims to enhance early diagnosis, improve treatment effectiveness, and establish more efficient monitoring methods, ultimately contributing to better care and outcomes for individuals affected by SMA.

SESSION 4: Developmental Aspects of SMA

Morgan Gazzola (O14)

Morgan Gazzola is a postdoctoral researcher in Cécile Martinat's team at the I-Stem laboratory in Paris. He started his career with a PhD in the field of airway hyperresponsiveness and airway smooth muscle mechanics in Canada in the team of Ynuk Bossé. After his thesis, Morgan carried out a first postdoctoral experience in the field of cytoskeletal organization and cell mechanics in the team of Manuel Théry and Laurent Blanchoin in Paris. Passionate about unravelling the key mechanisms involved in various pathologies and keen to develop new cellular models using stem cells, he joined Cécile Martinat's team in 2022. Today, by combining two- and three-dimensional approaches, he is trying to understand new molecular mechanisms involved in spinal muscular atrophy, but also in other neuromuscular diseases such as Steinert myotonic dystrophy.

Federica Genovese (O16)

Federica is a final year PhD student in Prof. Tom Gillingwater's lab at the University of Edinburgh, Scotland, UK. She was born in Turin, Italy, where she obtained her Bachelor's and Master's degrees in Molecular Biotechnology. During her studies she was selected to participate in a double degree programme in BioHealth Engineering at the Université Grenoble Alpes, in France. Experiencing this dynamic and highly varied field of study inspired her interest in a career in neuroscience and developmental biology. With the aspiration to be part of a thriving scientific community, in 2021 she moved to Edinburgh to pursue her PhD exploring the embryonic developmental aspects of SMA. As part of the SMABEYOND Consortium, funded by the European Union HORIZON 2020, Federica's research focuses on investigating systemic organogenesis and the molecular mechanisms that underlie SMA pathogenesis.Immersed in an international and vibrant scientific community, she recognises even more the importance of collaboration as a key driver of scientific progress. Federica is grateful to be part of the SMA scientific community and for the opportunity to present her work, with the hope of making a meaningful impact for the patients and their families.

SESSION 5: New avenues in SMA research

Ilaria Signoria (O17)

Ilaria received her BS in Biomolecular Sciences and Technologies and MS degrees in Molecular and Cellular Biotechnology from the University of Trento, Italy. During her master's program, she conducted the research internship in Gabriella Viero's lab, focusing on spinal muscular atrophy (SMA). Her master's thesis investigated translational defects in various models, tissues, and stages of SMA. In April 2021, she joined the SMA Centrum at the UMC Utrecht, Netherlands, as a PhD student under the supervision of Ludo van der Pol and Ewout Groen. Her PhD project, part of the EU-funded SMABeyond consortium, aims to identify molecular biomarkers to predict disease severity and treatment response in patients. This will help clinicians in giving more accurate prognosis and decide across treatments, moving towards a personalized approach.

Tobias Schüning (O18)

Tobias received his university entrance qualification 2012 at his birth´s place Schwerin, Germany and finished his bachelor´s degree in Biochemistry at the Leibniz University Hannover, Germany in 2016. He first worked on neuronal development and disease-associated proteins in the Universidad Nacional de Córdoba, Argentina, during an internship in his master studies. In 2018 Tobias finished his Master of Science in Biochemistry with the master thesis entitled 'Cell biology of post-translational modifications of the survival of motoneuron (SMN) protein' at the Prof. Peter Claus group, Hannover Medical School, Germany.

Further on, he stayed with the Claus group and participated in the PhD program 'Systems Neuroscience' at the University of Veterinary Medicine Hannover and was awarded with the degree of Doctor of Natural Sciences (Dr. rer. nat.) when he defended his doctoral thesis entitled 'Molecular mechanisms of cytoskeletal dysregulations in Spinal Muscular Atrophy (SMA)' in 2023. Currently, he is Scientist at the SMATHERIA gGmbH – Non-Profit Biomedical Research Institute in Hannover, Germany. He is interested in the cell-biology of dysregulated actin dynamics in SMA beyond the dominant motoneuron phenotype. This may lead to the identification of pathways not susceptible for SMN restoration and therefore development of combinatorial treatment approaches.

Saman Rashid (O19)

Saman graduated from the University of Hertfordshire with a BSc (Hons) in Biomedical Sciences before pursuing an MSc by research into the autophagy networks perturbed in spinal muscular atrophy. He is currently doing a PhD on a pharmacological approach to elucidating these perturbed networks.

SESSION 6: Muscles in SMA

Fiorella Grandi (O22)

My name is Fiorella Grandi, I am postdoctoral researcher at the Centre de Recherche en Myologie (INSERM UMR 974) in Paris, France. I completed by PhD thesis at Stanford University (California, USA) where I studied the skeletal system and how it develops. I then completed a first postdoc at the Gladstone Institutes studying neurovegetative diseases. I am specialised in the study of the epigenome -- the way cells chemically change the way they use their DNA based on developmental and environmental cues – and in particular in the application of "omics" technologies to patient-derived samples. I am now applying these techniques to understand the varied presentations of SMA and the various responses to approved therapies, particularly in peripheral tissues like the muscle, to better understand the heterogeneity of SMA and the remaining gaps in treatment.

SESSION 7: Monitoring disease outcome

Leandra Ros (O26)

Leandra Ros graduated from the University of Utrecht in 2018 with a degree in Medicine. During her time in medical school, she engaged in research focused on airway clearance techniques for children with neuromuscular diseases, specifically directing her attention to spinal muscular atrophy (SMA).
Following her graduation, she served as a Junior Doctor in pediatric medicine for 1.5 years, during which she gained exposure to pediatric neurology. This experience ignited her interest in integrating clinical practice with research.
In 2020, she embarked on a Ph.D. journey, exploring the application of various electrophysiological techniques in both pediatric and adult patients with SMA. Her research seeks to enhance our understanding of the (dys)function of the peripheral motor circuit in SMA and evaluate the effect of different treatment modalities. Moreover, her research extends to explore whether these techniques hold the potential to predict treatment outcomes for patients with SMA at the onset of their therapy.

Chiara Panicucci (O28)

I am a pediatrician specialized in neuromuscular disorders, with a great interest in clinical and translational research.
I am responsible of pediatric and adult patient's management in the outpatient unit directed by Prof. Claudio Bruno at IRCCS Giannina Gaslini Institute, where I am mostly dedicated to patients affected by Spinal Muscular Atrophy (SMA), Dystrophinopathies (DMD-BMD), Limb Girdle Muscular Dystrophies (LGMDs), and Glycogenosis.
My clinical activity ranges from patient's clinical evaluation to decisions on diagnostic approaches, to critical observation of disease progression by analyzing changes in functional, radiological, and serum/CSF biomarkers.
Since the Medical School, I developed a great interest in basic and translational research, focusing my interest on understanding the role of inflammation in the pathogenesis of muscular dystrophies. During my Residency in Pediatrics, I spent 1 year as Visiting Researcher at Laboratory of Molecular Medicine, University of Portsmouth, UK working on a pre-clinical project concerning the pro-inflammatory P2X receptors pharmacological inhibition in mdx mice (2016-2017).
Moreover, during my PhD program, I visited the Leibniz Institute Deutsches Rheuma Forschungszentrum (German Rheumatism Center, DRFZ), Berlin working on a pre-clinical project concerning the characterization of Innate Lymphocytes in mice models of muscular dystrophies (2019-2020).
Thanks to a close collaboration with biologists and bioinformaticians, I am currently involved in the discovery of CSF and serum biomarkers in SMA through a multi-omics approach.

PODIUM PRESENTATIONS

Rehabilitation in SMA - Motor neuron and spinal cord pathology

O1
Facilitating neuromuscular junction recovery after treatment in mouse models of Spinal Muscular Atrophy

I. Partlova[1,2], L.H. Comley[1,2], L.M. Murray[1,2]
[1]Centre for Discovery Brain Sciences, University of Edinburgh, Edinburgh; [2]Euan McDonald

Centre for Motor Neuron Disease Research, University of Edinburgh, Edinburgh, UK

Despite the availability of therapeutic options for SMA, impactful deficits persist in affected patients even after pre-symptomatic treatment. A hallmark of motor unit pathology in SMA involves the loss of motor neurons and disruption of neuromuscular junctions (NMJs). It is important to determine what deficits remain following treatment with a Smn enhancing compounds and how to develop combinational therapeutics to target such deficits.

Here we have analysed neuromuscular junction recovery following early (P2) administration of nusinersen in the SmnΔ7 mouse model. We have employed the mouse cranial muscles which facilitate whole mount analysis of a group of differentially vulnerable motor units. We also conducted efficiency screening to assess the impact of compounds with the potential to enhance regeneration and promote repair in SMA. These experiments were carried out in both the $Smn^{2B/-}$ and SMNΔ7 mouse models. The compounds were administered from postnatal day 3 (P3) until the mice reached their respective end-stage conditions.

Following nusinersen treatment, the majority of endplates were fully occupied. However, we observed an underlying loss of axons and endplates which was most profound in the most vulnerable cranial muscles (e.g. auricularis superior; AS). This work reveals important deficits in motor unit recovery following an increase in Smn levels. In attempt to target this persistent loss of axons, we have performed small scale screening using pro-regenerative drugs in both the $Smn^{2B/-}$ and SMNΔ7 mouse models and have identified test compounds which have the capacity to ameliorate NMJ pathology and enhance motor performance.

This study emphasises the regenerative potential of motor neurons following Smn restoration but stresses that the recovery is incomplete. These findings also highlight the potential of Smn independent compounds as a pro-regenerative agents in SMA and their significant clinical implications.

O2
Respiratory muscle training in patients with spinal muscular atrophy - Results of a randomized controlled trial

K. Kant-Smits[1], B. Bartels[1], C.K. van der Ent[2], E.S. Veldhoen[3], F. Asselman[4], R.P.A. van Eijk[5], W.L. van der Pol[4], H.J. Hulzebos[1]

[1]Child Development and Exercise Center, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht; [2]Dept. of Pediatric Pulmonology and Allergology, University Medical Centre Utrecht; [3]Dept. of Pediatric Intensive Care, University Medical Centre Utrecht, The Netherlands; [4]Dept. of Neurology and Neurosurgery, University Medical Center Utrecht; [5]Biostatistics & Research Support, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands

Respiratory muscle weakness is an important complication of Spinal Muscular Atrophy (SMA) and may result in impaired cough with recurrent respiratory tract infections, nocturnal hypoventilation, and ultimately in respiratory failure. Strategies to improve respiratory muscle strength are needed.

To assess feasibility (adherence, acceptability), and efficacy (respiratory muscle strength, lung function dyspnea, quality of life) of respiratory muscle training (RMT) in patients with SMA types 1-4 and respiratory muscle weakness.

We conducted a single blinded randomized sham-controlled trial consisting of a 4-month training period followed by an 8-month extension phase. The RMT consisted of a home-based, individualized training program involving 30-breathing cycles through an inspiratory and expiratory muscle training device. Participants were instructed to perform 10 training sessions over 5-7 days per week. The training group started the training with an intensity of 30% of their maximal inspiratory (PImax) and expiratory pressure (PEmax) and adjusted the training intensity based on their perceived exertion. The control group initially received RMT at the same frequency but against a constant, non-therapeutic resistance. After 4 months they received the same intervention as the training group (delayed intervention).

Three children and 27 adults were included. The mean age of the participants was 37.53 years. Three participants were diagnosed with SMA type 1, 19 with SMA type 2, seven with SMA type 3, and one with SMA type 4. Seven participants received no SMN-augmenting therapy, 17 received a maintenance dose of Risdiplam, and six of Spinraza□. The average PImax % of predicted (%pred) at baseline was 60.33% and PEmax %pred was 28.03%. In the first 4 months, the training- and control group performed, respectively, 62% and 91% of the prescribed RMT sessions, while all participants scored acceptability as ''good'' (≥5 on a 0-10 scale) after 4 months of training. There was no significant difference in PImax %pred between the training- and control group after 4 months of training (mean difference of 3.56% [95% CI: -8.75, 15.86]). However, the training group did show a significant increase in PImax %pred of 14.13% (p=0.003) from baseline to month 4. The control group showed a non-significant increase in PImax %pred of 9.6% (p=0.064). PEmax %pred slightly increased in the training group (4.87%, p=0.082) and in the control group (3.33%, p=0.256). Lung function, dyspnea and quality of life remained stable. There were no serious adverse events related to the training.

Respiratory muscle training is a feasible and probably effective method to improve inspiratory muscle strength in patients with SMA and is therefore a promising addition to current treatment for patients with respiratory muscle weakness. Future research should focus on finding the optimal training volume while maintaining good adherence.

O3

Bone health in adult spinal muscular atrophy (SMA) patients: Is it a forgotten risk?

C. Hewamadduma[1,2], L. Chapman[3], S. McNicholas[1], K. Nevin[2], Z. Cader[1], L. Maidment[2], A. Navalgaria[2], S. Packwood[2], S. Leighton[2], J. Street[1,2]

[1]Sheffield Institute for translational neurosciences (SITRAN), University of Sheffield; [2]Sheffield Teaching Hospitals Foundation NHS Trust, Sheffield; [3]Sheffield Children's Hospital, Sheffield, UK

Spinal muscular atrophy (SMA) due to loss of SMN1 gene causes progressive, symmetrical muscle weakness and muscular atrophy of the limbs, trunk, respiratory and bulbar muscles. Whilst type 1 SMA patients seldom survived to adulthood, SMA type 2 and 3 patients represent the majority of adult SMA clinics.

Patients with SMA are at a higher risk of decreased bone mineral density (BMD), increased incidence of osteopenia, osteoporosis, and fractures, regardless of age or type of SMA. A previous Smn-/-SMN2 mouse model highlighted that loss in SMN1 causes an increased rate of osteoclast formation and bone resorption capacity and up-regulation of the RANK receptor signalling molecules which causes osteoclast differentiation. Poor bone health puts SMA patients at an increased risk of fractures. SMA type 3 walkers could be at risk of falls and may struggle to recover ambulation post-fracture and Type 2 SMA patients risk fractures during transfer, hoisting and turning. Fracture prevention is particularly important to maintain ambulation in type 3 SMA patients.

We designed an audit tool to assess the bone health in a large single centre cohort of adult-SMA patients with a view to extending the audit to multiple UK centres. Audit tool was designed by the multi-disciplinary team. We included 58 SMA patients who attend the regional SMA centre in Yorkshire, Sheffield, UK. We collected demographics, phenotype, genotype, bone health biochemistry, outcome measures, DEXA scan and pharmacological management details and any interventions that have been conducted to prevent fractures.

Total of 53 out of 58 patients included had full data set. Type 2, n=25 and type 3, n=28, of which 13 were ambulant. Median age was 29.5 years. 31 patients were on Nusinersen and 22 on Risdiplam. DEXA scan was done in only in 31% of patients (SMA-Type 2 vs 3, 8% vs 54%, $p<0.0006$). Suboptimal vitamin D level was observed in 85%, whilst in 60% it was severely low. All SMA type 3 patients had low vitamin D. 3 out of 13 Type 3 ambulatory patients suffered a fracture resulting in loss of ambulation. Follow up DEXA scan results and impact of therapies on bone outcomes will be discussed in the presentation.

Our results highlight that there is significant room to improve bone health in SMA patients and fracture prevention is particularly important in SMA type 3 patients as it can lead to non-ambulation in adult SMA patients. Systemic therapy of mesenchymal stem cells has previously been found to promote bone regeneration in preclinical experiments. New therapies give us a renewed understanding of unmet needs and highlight poor bone health as an under-recognised comorbidity in the SMA patients and future care standards should address this requirement. Our audit tool has been validated and can be used by other centres to evaluate the bone health management in SMA patients.

O4

Scoliosis progression in spinal muscular atrophy type II: A comparative study between treated and untreated patients

G. Coratti[1], J. Lenkowicz[1], M.C. Pera[1], A. D'Amico[2], C. Bruno[3], C. Gullì[1], N. Brolatti[3], L. Antonaci[3], M, Ricci[1], A. Capasso[1], G. Cicala[1], R. De Sanctis[1], M. Catteruccia[2], L. Labianca[4] , A. Leone[1], S. Patarnello[1],
M. Pane[1], V. Valentini[1], E. Mercuri[1]

[1]Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome; [2]Ospedale Pediatrico Bambin Gesù IRCCS, Rome; [3]Ospedale Pediatrico Giannina Gaslini IRCCS, Genoa; [4]Sant'Andrea Hospital, Rome, Italy

Spinal Muscular Atrophy (SMA) is a debilitating disease that affects patients worldwide. While pharmacological therapies have shown to be effective in enhancing motor function in SMA patients, scoliosis management remains a significant challenge. The impact of these therapies on scoliosis is still uncertain, and it can have a negative impact on patient health outcomes. The objective of this prospective study was to examine the impact of pharmacological treatment on scoliosis progression in Type II SMA patients and compare it to untreated individuals.

The ISMAC centers in Italy conducted a retrospective analysis of prospective data encompassing patients with SMA types II from January 2013 to September 2023. During this period, patients were categorized into two groups: those who received treatment and those who did not, and they were observed for a minimum duration of 1.5 years. The analysis considered various patient characteristics, including motor function level, age, and sex, in addition to X-ray assessments while in a seated position. Changes in Cobb angle were calculated by measuring the total change over the follow-up period. The study aimed to investigate the impact of treatment on annual changes in Cobb's angle and the achievement of a 50° Cobb angle milestone in patients with Type II SMA, considering a minimum follow-up duration of 1.5 years. To determine the optimal treatment subpopulation, a sliding cut-off approach was employed, taking into account factors such as age, Cobb angle, and HFMSE scores at the initial visit. The statistical significance of the findings was assessed using the Mann-Whitney U-test.

Any significant difference was found in terms of age, SMN2 copies, SMA function, HMSFE score and Cobb angle at the baseline between untreated (n=46) and treated (n=39) population. Of these, 9/39 (23%) were on risdiplam and 30/39 (77%) on nusinersen. Scoliosis progression showed no significant differences between the two groups (p=0.4). Optimal cut-off values for a better outcome were Cobb angle <26° and age <4.5 years. In the resampled population based on these cut-off values, the untreated group had a mean Cobb variation of 10.05 (SD 6.38) degrees/year, while the treated group had 5.61 (SD 4.72) degrees/year (p=0.01). Cox regression analysis indicated a protective treatment effect in reaching a 50° Cobb angle, significant in patients <4.5 years old (p=0.016).

These findings suggest that early treatment with pharmacological therapies can not only improve motor function in SMA patients, but it can also modify scoliosis progression.
In conclusion, early treatment can optimize outcomes and modify scoliosis progression, emphasizing the critical role of timely action in managing SMA. Larger studies are warranted to further investigate the effectiveness of individual pharmacological treatment on scoliosis progression in this patient population.

Optimising Gene-directed Therapeutics

O5
RNA perturbation in Cerebrospinal Fluid of Spinal Muscular Atrophy Patients treated by Nusinersen

F. Dragoni[1,2], R. Di Gerlando[1,2], B. Rizzo[1], M.I. Dainesi[3,1], E. Scarian[1], A. Iosca[3,1], M. Bordoni[1], S. Parravicini[3,1], O. Pansarasa[1], A. Berardinelli[1], Gagliardi S.[1]

[1]IRCCS Mondino Foundation, Pavia; [2]Dept. of Biology and Biotechnology, University of Pavia, Pavia; [3]Dept. of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy

Spinal muscular atrophy (SMA) is a rare autosomal-recessive neurodegenerative disorder caused by mutations in survival motor neuron 1 (SMN1) gene, and consequent loss of function of SMN protein, which results in progressive loss of lower motor neurons, and muscular wasting. Antisense oligonucleotide (ASO) nusinersen (Spinraza®) modulates the pre-mRNA splicing of the SMN2 gene, allowing rebalance of biologically active SMN. It is administered intrathecally via lumbar puncture after re-moving an equal amount of cerebrospinal fluid (CSF). Its effect was proven beneficial and approved since 2017 for SMA treatment. Given the direct effect of nusinersen on RNA metabolism, the aim of this project was to evaluate cell-free RNA (cfRNA) in CSF of SMA patients under ASOs treatment for biomarker discovery.

By RNA-sequencing approach, RNA obtained from CSF of pediatric SMA type 2 and 3 patients was processed after 6 months of nusinersen treatment, at fifth intrathecal injection (T6), and compared to baseline (T0).

We detected 48 deregulated genes (DE) cfRNAs of which 36 mRNAs, 1 lncRNA and 11 “other bio-type” RNAs (processed pseudogenes, unprocessed pseudogene and untranscribed unprocessed pseudogenes). We found 23 down-regulated and 13 up-regulated mRNAs, the only lncRNA was down-regulated, while 7 “other biotype” RNAs were up-regulated and 4 were down-regulated. Gene ontology (GO) analysis showed an involvement of numerous pathways associated to splicing such as regulation of mRNA splicing, spliceosome and regulation of mRNA processing related to SAP18 gene alteration. Also, GTPase genes have been found altered and they are well known to be implicated in skeletal muscle development and regeneration. In fact, the expression of GTPases is critical for skeletal muscle differentiation and can regulate the expression of MyoD and myogenin which dysregulation is associated to the muscle weakness observed in SMA.

This study provides preliminary insights into modulation of gene expression dependent on nusinersen treatment and lays the foundation for biomarkers discovery. Our data open to new studies to better understand of the discussed molecular pathways in SMA pathogenesis and their modulation after SMN2-targeting.

O6
Direct evidence of deletion and gene conversion as origin of SMA alleles revealed by long-read sequencing

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The spinal muscular atrophy (SMA)-causing gene *SMN1* and SMA-modifying gene *SMN2* are located in the highly complex *survival motor neuron (SMN)* locus. Traditional sequencing and bioinformatic methods are insufficient to understand the full scale of genetic variation in this locus, and how it is associated with disease severity and treatment outcomes. According to current consensus, *SMN1* can either be deleted or converted into *SMN2*, giving rise to an SMA allele lacking *SMN1*. However, this theory is mostly supported by indirect evidence such as copy number of *SMN1/2* and neighbouring genes. Here, we aimed to determine the structure of the *SMN* locus in SMA patients to provide direct evidence for the deletion and gene conversion theory and to investigate structural variation as a possible disease modifier. We isolated high molecular weight DNA from blood or fibroblasts of 32 patients with *SMN2* copy numbers ranging from two to five and performed amplification-free, targeted long-read Nanopore sequencing of the *SMN* locus. Sequencing data was mapped to a reference genome in which *SMN2* and surrounding genes have been masked to avoid ambiguous mapping. Polyploid variant calling and haplotype phasing was performed based on the predetermined *SMN2* copy number. We were able to distinguish different *SMN2* copies based on structural and sequence variation. The 200 kilobase-long genomic environment, both upstream and downstream of *SMN2*, varied greatly: we identified *SMN* locus configurations where *SMN2* was surrounded by genes typical for an *SMN1* environment in 14% of resolved haplotypes, indicative for gene conversion events. Hybrid combinations with an upstream *SMN2* environment and downstream *SMN1* environment or vice versa were detected in 28% of resolved haplotypes, suggesting that recombination and gene conversion not only occurred between *SMN1* and *SMN2*, but also between other genes in the locus. Three patients with no *SMN1* environment were also identified, supporting the *SMN1* deletion theory. Interestingly, one of these patients had three *SMN2* copies but no *SMN1* environment, possibly indicating a homozygous *SMN1* deletion and a recent duplication of *SMN2*. In conclusion, we here provide direct evidence for the deletion and gene conversion theory by showing the structure of the *SMN* locus in SMA patients. Varying environments of the *SMN2* gene could have direct implications for gene expression due to changes in regulatory sequences or DNA methylation patterns, providing a possible explanation for genotype-phenotype discordances and varying treatment response in SMA patients.

O7

Characterizing the effect of AAV9 mediated gene therapy for SMA on the epigenetic and transcriptional stability of SMA mice

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Adeno-associated virus (AAV) based gene therapies are poised to have a large impact on rare diseases. ZOLGENSMA treatment has been life altering for spinal muscular atrophy (SMA) Type I infants treated perinatally, with incredible improvements in life expectancy and gain of developmental milestones. While this therapy is clearly life changing, it also highlights the importance of understanding how AAV-mediated gene therapies are regulated after injection, especially in the case of childhood diseases that require stable and reliable gene expression over a lifetime. The epigenetic landscape, which consists of chemical modifications to DNA and histones, ensures the correct temporal regulation of gene expression–and changes to the epigenetic landscape have been found to contribute to many diseases. However, the effect(s) of gene therapies like ZOLGENSMA on the long-term stability of the epigenome is unknown.The goal of this work is to understand how the epigenome reacts to ZOLGENSMA administration and to use this information to create better gene therapies.

SMAd7 mice were treated with $5^{E}10$vg viral genomes/kg at postnatal day 1 using intraventricular injection. DNA and RNA were extracted from matched tibialis anterior muscles and used as the input for library generation. The 5hmC libraries were generated using the Zymo's Reduced Representation Hydroxymethylation profiling kit (RRHP). Differential CCGG methylation status was called using DeSEQ2.

We had previously shown that ICV injection of AAV9-PGK-SMN1 resulted in efficient transduction of the brain, spinal cord, muscle and other peripheral tissues, with a viral genome copy number/ nucleus of ~40 in the spinal cord and muscle, allowing us to study the effect AAV therapy in these tissues. To study the epigenetic landscape, we began by profiling 5-hydroxymethylcytosine (5hmC) location and chromatin accessibility of treated and untreated *tibialis anterior* muscle. 5hmC is a chemical modification to the cytosine base, whose accumulation at enhancers and gene bodies allows for gene expression. Thus, by profiling the 5hmC landscape in combination with RNA-sequencing, we can observe genes which are currently active (5hmC + RNA expression) and those that will be activated (5hmC only). As expected, we observed vast transcriptional and 5hmC differences between SMA and WT muscle. In line with our physiological data, gene therapy restored the transcriptome of the SMA mice at postnatal day 14 and most of the SMA-disease associated 5hmC changes were restored by the AAV therap at an early timepoint, in line with the restoration of the transcriptome. However, the 5hmC profile of the injected mice also contained new changes not observed in the WT or SMA mice, suggesting that these 5hmC-changes may result from the AAV injection. Indeed, we observed similar changes in WT AAV9-GFP injected mice.These 5hmC alterations affected genes involved in innate immune activation, apoptosis, and metabolism. Ongoing work is tracing these effects long-term (4.5 and 6.5 months after mice treatment), when variable responses to gene therapy in our animal cohort are evident.

Preliminary results suggest that while gene therapy ensures short term transcriptional restoration, many aspects of the epigenetic landscape are not fully restored and they are predictive of the variable long-term response. Ongoing work is testing the effect of the route of injection, the capsid type and the promoter used for expression on these parameters, as well as determining the molecular mechanisms that explain the heterogeneity in treatment response.

O8

Enhancing In Vitro SMN Protein Expression and Cell Viability through Xeno-Nucleic Acid-Based ASOs in Spinal Muscular Atrophy

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Spinal Muscular Atrophy (SMA) stands as a devastating ailment arising from the dearth of functional SMN (Survival Motor Neuron) protein due to genetic anomalies within the SMN1 gene. This condition is marked by the consequential attrition of motor neurons, precipitating a progressive decline in muscular strength and culminating in the disruption of neuromuscular junctions. Existing therapeutic approaches encompassing Zolgensma, Nursinersen, and Evrysdi employ innovative genetic therapeutic strategies involving transgene delivery, Antisense Oligonucleotide (ASO) technology, and modulation of pre-mRNA processing to enhance functional SMN protein expression. However, the ASO therapeutics remain suboptimal in establishing a sustained panacea for SMA, as they inadequately maintain consistent levels of functional SMN protein expression. In this study, we present a discerning inquiry into focusing on XNA-DNA-ASO products that exhibit enhanced safety and stability compared to conventional DNA/RNA-ASO sequences. Through precise targeting of the ISSN-1 region within SMN2 gene's intron 7, our approach seeks to amplify SMN protein expression. Employing Xeno Nucleic Acid (XNA) bases, known for their augmented hydrophobicity and stability, our strategy surmounts previous limitations associated with chemical modifications, showcasing heightened endonuclease resistance. Comparative analyses with conventional DNA/RNA-ASO products substantiate the superiority of XNA-DNA-ASO sequences, underscoring elevated SMN protein expression and reduced toxicity. In a comprehensive evaluation, our gene therapy paradigm is scrutinized within a type 1 SMA fibroblast cell line. Utilizing diverse analytical methodologies, encompassing Annexin V-PI analysis for cytotoxicity, MTT assays for mitochondrial activity, and flow cytometry for SMN protein expression profile, we gauge therapeutic impact and potential toxicity. In conclusion, our investigation not only spotlights the promise of XNA-DNA-ASO sequences but also holds implications for refining SMA treatment strategies, converging on minimized dosages, lowered toxicity, and heightened therapeutic efficacy, thus shaping the landscape of gene therapy for SMA.

In conclusion, the results of this study contribute to the ongoing quest for improved therapeutic interventions for SMA. By harnessing the advantageous properties of XNA-DNA-ASOs, we present a compelling case for the sustained enhancement of SMN protein expression and cellular viability. These findings open up new avenues for the development of next-generation genetic therapies that offer long-term stability and improved outcomes for individuals affected by SMA.

SMN beyond the Motor Neuron

O9
Selective loss of excitation and p53 activation cause cerebellar circuit pathology in spinal muscular atrophy

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Spinal muscular atrophy (SMA) is caused by a ubiquitous reduction of the SMN protein and is characterized by the degeneration of spinal motor circuits resulting in impaired voluntary movement and muscle atrophy. The contribution of brain motor circuits to SMA pathology is largely unknown. The motor circuits in the cerebellum are critical for motor learning and voluntary movements by processing proprioceptive input and modulating motor output, both of which are affected in SMA. A few studies reported alteration of Purkinje cells (PC) - the sole functional output of the cerebellar cortex - in SMA patients, suggesting a possible involvement of cerebellar pathology in the disease. Here, we investigated the extent and mechanisms of cerebellar pathology in SMA patients and mouse models.

We performed immunofluorescence, confocal and super-resolution microscopy on sagittal vermis sections from the cerebellum of Taiwanese and *SMNΔ7* SMA mouse models. In addition, we investigated the PC number in the cerebellum of control and SMA patients Type 1 by confocal microscopy.

Our results showed a significantly underdeveloped cerebellum including a reduced size of all cerebellar layers and PC lacking dendritic trees in *Taiwanese* SMA mice. *SMNΔ7* SMA mice also exhibited a slightly reduced cerebellum size and selective PC death in specific cerebellar lobules. Importantly, a reduction in PC number was also found in the cerebellum of SMA patients compared to control patients. Further analysis in *SMNΔ7* mutant mice revealed smaller PC dendritic trees and reduced excitatory synaptic input onto PC, indicating decreased activation of PC by granule cells and the inferior olive in SMA. Remaining excitatory synapses revealed a post-synaptic reduction of glutamate receptors, consistent with impaired cerebellar circuitry. To gain insight into the pathomechanisms, we investigated the p53 pathway which contributes to motor neuron death in SMA. Importantly, vulnerable PC exhibited robust p53 upregulation prior to their death and viral-mediated knockdown of p53 prevented PC death, demonstrating p53-dependent neurodegeneration in the cerebellum. Interestingly, viral-mediated SMN restoration prevented PC death but did not fully correct cerebellar circuit pathology, indicating a limited therapeutic benefit within the cerebellum.

Our results reveal cerebellar circuit pathology comprising of selective PC death and reduced excitatory synaptic input in both SMA mouse models and human patients, pointing to the cerebellum as a potential contributor to motor dysfunction in SMA. Viral-mediated SMN therapy does not correct circuit dysfunction due to the exclusive transfection of PC within the cerebellum.

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O10
Microvascular dysregulation: the missing link in SMA pathogenesis?

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Spinal muscular atrophy (SMA) is a rare paediatric motor neuron disease, which if untreated is a leading cause of infant morbidity and mortality. In addition to established neuromuscular pathology, depletion of cell-ubiquitous survival motor neuron protein also results in widespread non-neuronal pathology, especially within the cardiovascular system. Extensive microvascular pathology is described in SMA mouse models, with recent work uncovering intrinsic endothelial defects which may affect distribution, morphology and function of vessels. Microvascular dysfunction in the spinal cord has the potential to play a crucial role in neurodegeneration, through induction of a toxic neural environment and exacerbation of neuronal death. In addition, a damaged endothelium may lead to a weakened blood spinal cord barrier (BSCB). The spinal cord microvasculature may therefore be a significant factor in disease progression, contributing to neurodegenerative pathology in SMA patients.

In post-mortem, severe untreated SMA patients and age-matched control spinal cord, we quantified Von Willebrand Factor (VWF) labelled vessel profiles using novel stereological techniques and density measurements. Ki67 labelling generated an endothelial proliferation index. Electron microscopy was used to assess vessel and BSCB ultrastructure. Post-mortem samples were obtained from Johns Hopkins University, NIH Neurobiobank and BRAIN-UK.

It was immediately apparent that the microvasculature of the SMA spinal cord at end-stage was distinctly different to that of controls. The SMA spinal cord was populated with small and abnormal vessels, which had a universal reduction in VWF expression. In longer-surviving SMA patients, the density of these small vessels was increased, and this was most prominent in the grey matter. Increased endothelial cell proliferation suggested that ongoing, abnormal vessel growth contributed to this increased density. At the ultrastructural level, micro-vessels showed evidence of damage with ruptured endothelial plasma membranes, resulting in a loss of cellular integrity and cytoplasmic oedema. Gaps between adjacent endothelial cells allowed breach of the primary BSCB layer. Defects in the basal lamina and astrocyte end-feet showed an accumulation of pathology and suggested early impairment of the BSCB.

Vascular supply of the spinal cord is pivotal in controlling the neural environment. Vascular dysfunction has the capacity to exacerbate motor neuron pathology and negatively impact patient outcomes. We show that SMA patient spinal cord microvasculature is aberrant, with extensive micro-vessel and BSCB pathology, which correlates with existing patient blood biomarker data. While treatment options are available, it remains unknown to what extent extra-neuronal pathology will be targeted or treated. Targeting the vascular system may offer novel therapeutic opportunities in the treatment of SMA.

O11

Translational defects in multiple tissues from the Smn$^{2B/-}$ mouse model of SMA

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Studies across a range of animal and cellular models of SMA have deepened our understanding of the diverse roles of the Survival of Motor Neuron (SMN) protein, depletion of which is the root cause of SMA. FDA/EMA approved SMN-boosting therapies result in improved survival rates in human SMA patients but have also created a need for early diagnosis and treatment, as well as improved disease monitoring. Recent discoveries, such as the interaction between SMN and ribosomes/polysomes and the identification of "SMN-primed ribosomes" and their target mRNA transcripts termed "SMN-specific transcripts", have revealed that deficiency of SMN protein disrupts these interactions, resulting in translational defects in the target mRNAs during the pre and early stages of disease in the 'severe' Taiwanese SMA mouse. In this study, we have extended these investigations in order to ask whether translational defects can also be detected in different tissues from the milder Smn$^{2B/-}$ mouse model in order to establish whether translational defects are common across multiple mouse models. We also wished to validate these findings from mouse models in patient-derived fibroblasts from type I and type II SMA.

Utilising two complementary techniques, namely ribosome and polysome profiling, we examined changes in ribosome occupancy of mRNAs in brain, spinal-cord, and liver from the Smn$^{2B/-}$ mouse model at post-natal day 10. We validated these findings by comparing transcriptional, translational and protein levels by qPCR, co-sedimentation profiling, and western blotting at various disease stages and in patient-derived fibroblasts.

In brain, spinal cord, and liver, we observed a reduction in the fraction of ribosomes in polysomes in Smn$^{2B/-}$ mice along with loss of SMN from polysomes, consistent with previous observations in the more severe mouse model of SMA. By conducting ribosome profiling on central nervous system and in liver tissues at post natal day 10, we identified a set of genes enriched with extracellular matrix (ECM) transcripts, some of which are "SMN-specific" or a target of "SMN-primed ribosomes". One such "SMN-specific mRNA" is Col1a1, the most abundant ECM protein, which exhibited decreased association with polysomes in the absence of changes in mRNA abundance. This decrease was also evident at the protein level during early and late symptomatic stages of disease. Our observation was validated in the patient-derived fibroblasts from type I and II SMA patients, with more pronounced changes observed in the SMA type II cells compared to those from type I patients.

Our *in vivo* investigation of the Smn$^{2B/-}$ mouse model of SMA confirmed that defective association of SMN proteins with ribosomes/polysomes causes translational defects in a subset of transcripts and that these defects are mouse-model independent. The identification of early translation-specific defects in Col1a1 in SMA mice and patient-derived fibroblasts may open up new options for the use of translation-based biomarkers as well as new insights into the role of translation in disease pathogenesis.

O12

Identification of autonomic cardiovascular dysfunction in adult SMA patients: Towards the understanding of multisystem involvement in SMA

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Systemic pathology is emerging in spinal muscular atrophy (SMA) with the advent of new disease-phenotypes due to survival motor neuron protein (SMN)-rescue therapies, which are modifying SMA natural history. Previous reports of a defective autonomic nervous system (ANS) in preclinical models of SMA and severely-affected patients suggested an involvement of the ANS in the disease, which remains to be elucidated.

In this single center observational study, we aimed to evaluate whether adult SMA patients present a cardiovascular autonomic dysfunction.

Genetically defined adult (>18 y/old) SMA patients underwent cardiovascular autonomic tests (CATs) (10 min head-up tilt after 10 min supine rest, Valsalva manoeuvre (VM), deep breathing, sustained handgrip, cold face test, mental stress), and the COMPASS-31 autonomic assessment. Heart rate variability (HRV), baroreceptor sensitivity, sympatho-vagal balance, were continuously evaluated during the CATs. Results were compared with aged and sex matched controls and normative data using two-sample Wilcoxon rank sum test (Mann–Whitney) test.

Two type-2 and 14 type-3 adult SMA patients (6 sitters and 10 walkers) were enrolled. Cardiovascular reflexes indices were significantly more affected in SMA population compared with healthy subject, with a peculiar pattern characterized by increased bradycardic drive during deep breathing ($p<0.00002$), increased HR and variability of blood pressure during rest ($p<0,0001$), and head up tilt test ($p<0.0001$) consistent with a baroreflex afferent branch hypofunction and unbalanced sympatho-vagal. Moreover, we found an adrenergic hypofunction during VM ($p<0.0001$). Finally, SMA patients resulted symptomatic compared to controls ($p<0,0001$), as stated by The COMPASS score, particularly for othostatic intolerance ($p<0,0001$), gastrointestinal ($p<0,05$) and pupillomotor ($p<0,0001$) problems. No differences between sitters and walkers were identified. No correlations of the COMPASS score and cardiovascular parameters with: 1. degree of clinical severity measured by Hammersmith Functional Motor Scale-Expanded; 2. disease duration; 3. therapy duration; 4. time to therapy, were detectable.

Our data suggest the presence of autonomic cardiovascular dysfunction in adult SMA patients, probably related to impaired inhibitory drive on sympathetic outflow and sympato-vagal unbalance, contributing to properly define SMA phenotypic expression. Detecting ANS alterations may become more important if new therapeutic strategies will improve functional abilities and increase stress on the cardiovascular system. It might be also relevant to take these evidences into account for optimal clinical success of the therapeutic approaches.

Developmental aspects of SMA

O13
Time-course characterization of cortical projection neuron's alterations and development in delta7 SMA mice

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It has been well established that, in Spinal Muscular Atrophy (SMA), the lack of functional SMN protein, due to the mutation/deletion in the survival motor neuron (*SMN1*) gene, leads to the degeneration of spinal motor neurons (MNs), resulting in progressive muscle denervation and atrophy. Recent research in animal models and human patients has revealed that the brain is also impacted by SMN deficiency. Notably, studies on SMA models indicated changes in cerebral and cerebellar neural networks, while imaging studies on patients suggested, for instance, a reorganization of the cortical grey matter due to the ongoing degeneration of lower MNs. However, the cortical alterations in SMA and their involvement in disease progression still need to be clarified.
Therefore, we focused on studying the sensorimotor cortex of SMAΔ7 mice, as a severe model of SMA, with the goal to examine how a deficiency of SMN protein can affect the survival and organization of cortical projection neurons in time.

We conducted our analysis on early (postnatal day 4/5; P4/5) and late (P11) symptomatic SMA mice, compared to healthy WT littermates. By performing immunofluorescence analysis for various markers to distinguish different neuronal types, we investigated the number and distribution of projection neurons, and by using retrograde tracers we performed some morphological analyses. To look whether SMN depletion can affect projection neurons since cortical development, we performed thymidine-analogues (EdU, BrdU) injections in the dams at different embryonal time points and observed the labelled cell distribution in the cortical layers of pups.

In general, we observed changes in the structure of the cortex in SMA brain compared to WT with a decrease in cortical neurons. Specifically, when examining specific subtypes of projection neurons, at P11 we observed a reduction of about 40% of both corticospinal (Ctip2-positive) and callosal (Satb2-positive) cells in SMA cortex, together with a reduction of vGlut1 signal of about 38%. Although the analyses in early symptomatic brains (P5) suggest that these changes occur concurrently with spinal MN death and are not manifested earlier (with a reduction of cortical projection neurons by only 14% in SMA mice compared to WT), the evaluation of cell birth dating and distribution by thymidine analogues and other markers are helping us in revealing possible alterations in SMA cortex already at developmental stages.

Understanding the involvement of the cerebral cortex in SMA pathogenesis will aid in the comprehension of the mechanisms behind the disease progression, as well as in developing effective treatment approaches, beyond the spinal MNs, to improve clinical results.

O14
Transcriptional Mechanisms Differentiating Resilient and Vulnerable Spinal and Cranial Motor Neurons from human pluripotent stem cells in SMA

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Somatic motor neurons exhibit specific vulnerability in spinal muscular atrophy (SMA), even though the disease is caused by a deficiency of the survival of motor neuron protein, which is present throughout the body. In SMA, spinal motoneurons are highly vulnerable, yet certain motor neuron groups, such as cranial motoneurons that innervate facial and ocular muscles, remain inexplicably unaffected. Understanding the mechanisms underlying this selective vulnerability is therefore crucial to determine how the loss of a ubiquitously expressed protein can induce degeneration in a select cell type. This understanding, in turn, might guide the exploration of novel therapeutic pathways. However, investigating these mechanisms has been challenging due to limited access to these cell types in human.

To overcome this obstacle, we utilized human pluripotent stem cells (hiPSCs) to generate homogenous populations of spinal and cranial motoneurons that harbor a decreased SMN expression. While the connectivity to muscular targets and the survival of SMA hiPSC-derived spinal motoneurons were affected, SMA hiPSC-derived cranial motoneurons are notably preserved. A comparative transcriptional analysis revealed distinct misexpression of various synaptic genes in SMA hiPSC-derived spinal motoneurons, potentially explaining their compromised connectivity. These findings collectively underscore the potential to replicate the selective vulnerability associated with SMA in vitro, paving the way for the identification of novel therapeutic strategies aimed at preserving susceptible motoneurons.

O15

Isogenic patient-derived organoids reveal early neurodevelopmental defects in spinal muscular atrophy initiation

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Whether neurodevelopmental defects underlie selective neuronal death in neurodegenerative diseases is an intriguing hypothesis only recently explored. Spinal muscular atrophy (SMA) represents an ideal disease in which to test it given its early onset nature. In the most severe forms of SMA, recent studies have described abnormalities in motor neuron (MN) progenitors potentially associated to the reported defective MN migration, motor axon formation and outgrowth and MN target innervation and survival. Neurogenesis defects in several brain areas have also been observed in SMA model organisms.

To examine the potential role of neurodevelopmental defects at the basis of the selective MN loss in SMA, we created a novel in-vitro model using, for the first time, isogenic human induced pluripotent stem cell lines derived from patients with different SMA severities. Using a knock-in, CRISPR/Cas9 and vector-based genome editing approach, we corrected the nucleotide transition in one SMN2 locus responsible for the exon 7 skipping in 3 SMA hiPSC lines and generated two isogenic corrected clones per line. Further, our targeting vector introduced the green fluorescent protein Clover downstream of the corrected SMN2 exon 7, which allowed the visualization and tracking of SMN protein in live-imaging experiments. Added to these technological advances, we developed a novel spinal cord organoid (SCO) system to study potential early developmental alternations in spinal MN specification.

Our results indicate that SMA hiPSCs present a deficient capacity to self-assemble into stem cell aggregate structures and SMA SCOs exhibit abnormal morphological development, phenotypes that are notably ameliorated in the isogenic corrected clones. Additionally, the characteristic progressive death of SMA MNs is rescued in the corrected isogenic lines. Interestingly, we found a severely reduced expression of early neural progenitor and spinal cord-ventral progenitor markers and time-shifted expression of MN progenitor and MN markers in SMA SCOs, suggesting defective differentiation programs that may render specific MN subtypes to degenerate in their postnatal life. Furthermore, longitudinal single-cell RNA-sequencing revealed marked defects in neural stem cell specification and a reduced number of MNs, in favour of mesodermal progenitors and muscle cells. Surprisingly, converting SMN2 into SMN1 via genome editing was insufficient to revert all identified developmental abnormalities.

Taking advantage of our advanced system and a SCO model that we have newly developed, we unraveled that SMN deficiency leads to developmental abnormalities at the stem cell and neural progenitor level, which could sensitize specific MN populations to succumb later as the disease progresses. These alterations were only partially amended in the corrected isogenic clones. Our findings indicate that neuromesodermal developmental defects might underlie MN degeneration at later stages and suggest that interventions aimed at increasing SMN postnatally might not fully amend SMA pathology in all patients. Early combinatorial SMN-dependent and independent therapies might therefore be the best path forward to treat patients with SMA.

O16

Is SMA a developmental disease?

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Spinal Muscular Atrophy (SMA) is a childhood motor neuron disease that still does not have a cure. Despite the undeniable success of existing therapies in improving the outcomes and quality of life for SMA patients, there are still unanswered questions that need to be addressed. One of the most crucial challenges in SMA treatment is defining the timing of intervention. When treatment is administered as early as possible, ideally before symptom onset, patients obtain greater therapeutic benefits. Therefore, identifying the ideal timing for SMN restoration is key, as is understanding the underlying biological reasons. Recent work from our lab has revealed that SMN depletion leads to a broad spectrum of morphological and molecular perturbations in SMA mouse embryos, long before symptoms appear. SMN has also been shown to be a ribosome-associated protein with a critical role in translation and ribosome biology. SMN-primed ribosomes associate with specific mRNAs that are functionally related and have been linked to SMA pathogenesis. However, whether SMN's role in regulating translation and ribosomal biology plays a role in pre-natal development has yet to be investigated.

Our aim is to deepen the understanding of embryonic manifestations of SMA by characterising organogenesis in SMA mice *in vivo*. Here, specifically we investigated molecular mechanisms underlying embryonic SMA pathogenesis, with a particular focus on translation.

To assess the extent to which SMN-dependent defects in protein translation underpin pre-natal developmental changes observed in Taiwanese SMA mouse embryos at embryonic day 14.5 we performed ribosome profiling of brain, spinal cord and liver.

The data revealed a widespread organ-specific disruption in translation in SMA mouse embryos. Furthermore, we were able to identify key developmental pathways that are dysregulated when SMN levels are lowered to pathological levels.

Our results show that SMN is required for normal protein translation during embryonic development, and that SMN depletion leads to organ-specific defects in key developmental pathways.

O17

Molecular and morphological characterization of primary patient-derived fibroblasts to study gene-targeting therapies for spinal muscular atrophy (SMA)

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Our current ability to study the causes of the pronounced heterogeneity in disease and treatment outcomes in spinal muscular atrophy (SMA) remains limited. This is due to a lack of disease models that adequately capture this heterogeneity. For example, unlike patients, the most commonly used mouse models of SMA are genetically extremely homogenous. Moreover, induced pluripotent stem cell (iPSC)-based models provide the opportunity to include patient genetic variation, but their scalability is often limited due to costly and labour-intensive culturing protocols. Primary patient-derived fibroblast cell lines, in contrast, are easily obtained, cultured and expanded, and can be used to analyse key features of SMA *in vitro*. This, however, has not previously been done at a significantly large scale and with the goal to study disease and treatment variability. Here, we therefore characterised 45 primary fibroblast cell lines, including 35 SMA patients (type 1 = 9, type 2 = 15, type 3 = 7, type 4 = 4) and 10 control donors. We determined SMN protein, *SMN2-FL* and *SMN2-Δ7* mRNA expression and cellular morphology using semi-quantitative fluorescent western blotting, droplet digital PCR and fluorescent microscopy. In addition, we analysed the effect of *in vitro* treatment with *SMN2*-splicing modifiers nusinersen and risdiplam on these variables. We found that, morphologically, fibroblasts from SMA patients were comparable to controls, although nuclear size varied depending on *SMN2* copy number. Similarly, there was a significant but limited correlation between SMN protein and mRNA levels, and between *SMN2* copy number and protein and mRNA levels . After treatment, we observed a complete depletion of *SMNΔ7* mRNA, which led to a variable increase of *SMN2-FL* and SMN protein levels. Using a multiple linear regression model, we found that younger age, higher *SMN2* copy number and higher pre-treatment *SMNΔ7* and SMN protein levels were main predictors of *in vitro* treatment efficacy. In summary, we here highlight the use of patient-derived primary fibroblasts as a potential model to study heterogeneity of SMA disease and treatment outcomes.

O18

The Spinal Muscular Atrophy gene product regulates actin dynamics

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Survival of Motoneuron (SMN) protein loss preferentially affects α-motoneurons leading to their degeneration. Previously, we and others have shown that SMN directly interacts with the neuronal actin-binding protein profilin2a thereby influencing actin dynamics. Dysfunctional actin dynamics caused by SMN loss disrupts neurite outgrowth, axonal pathfinding, formation, and maturation of functional synapses in neurons. Whether SMN directly interacts with and regulates filamentous (F-) and monomeric globular (G-) actin is still elusive.

We used a quantitative single cell approach to measure F-/G-actin fractions and cellular morphology in our CRISPR/Cas modified motoneuron-like NSC34 cells which resemble reduced full-length SMN protein levels as seen in SMA. Furthermore, we assessed co-localization and co-immunoprecipitation of the SMN protein with both actin species, F- and G-actin in WT and SMA like cells. To further delineate the putative direct protein-protein interaction we conducted *in vitro* pull-down experiments with recombinant F-/G- actin and SMN.

We demonstrate that SMN loss leads to dysregulated F-/G-actin fractions correlating with changes in cellular morphology of our motoneuron-like cell line. Furthermore, correlating changes in actin dynamics with the distinct morphological alterations, these results suggest an F-actin organizational defect. Interestingly, this is mediated by a direct interaction of SMN with G- and F-actin as shown by i*n vitro* pull-down experiments. In co-immunoprecipitation and co-localization assays we elucidated that this interaction is independent of the SMN-profilin2a interaction. Therefore, we suggest two populations being relevant for functional actin dynamics in healthy neurons: SMN-profilin2a-actin and SMN-actin. Additionally, those two populations may influence each other and, therefore, regulate binding of SMN to actin. In SMA, we showed a dysregulated co-localization pattern of SMN-actin which could only partially be rescued by SMN restoration.

Taken together, our results suggest a novel molecular function of SMN in binding to F- and G-actin independent of profilin2a. Three drugs for treatment of SMA are approved in the US and Europe. They restore SMN protein levels and in most cases partially ameliorate disease phenotypes. Therefore, combinatorial treatment options are needed to further improve clinical outcomes. Here we show that dysregulated actin dynamics in SMA becomes SMN-independent at a particular developmental stage, underlining the importance of combinatorial treatment options apart from SMN restoration.

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O19

Autophagy in spinal muscular atrophy: Detrimental or a therapeutic avenue?

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Spinal Muscular Atrophy (SMA) is an autosomal recessive neuromuscular disorder characterised by the degeneration of α-motor neurons in the anterior horn of the spinal cord, resulting in progressive muscle loss and ultimately death. SMA is caused by reduced levels of the ubiquitously expressed survival motor neuron (SMN) protein. Autophagy is a highly conserved lysosomal degradation pathway known to be associated with neurodegenerative disorders such as Alzheimer's, Parkinson's, Huntington's and amyotrophic lateral sclerosis. When SMN levels are depleted, autophagy is dysregulated suggesting a potential role in SMA pathogenesis. Nevertheless, findings from mammalian and cell culture studies remain highly controversial. We aim to define the specific autophagy networks dysregulated in SMA using the well-established *Caenorhabditis elegans* (*C. elegans*) SMA model. We initially challenged the nematode pharmacologically by exposing animals to modulators of autophagy which are known to affect varying stages of the pathway. We found that autophagy modulators were capable of ameliorating SMN neuromuscular defects by increasing pharyngeal pumping, an indicator of neuromuscular function. Next, we observed the effects of the aforementioned compounds on animal mobility and found a number of behavioural parameters that increased locomotory activity. Furthermore, we identified that modulation of the autophagic pathway also resulted in increased animal lifespan. Our results suggest that modulation of varying steps in the autophagic machinery (a) ameliorates SMN neuromuscular defects and (b) improves animal survival. We are currently addressing the mechanism of action underpinning the results obtained in order to decode the role of autophagy in SMA pathology.

O20

RAINBOWFISH: Primary efficacy and safety data in risdiplam-treated infants with presymptomatic SMA

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Risdiplam (EVRYSDI®) is a centrally and peripherally distributed, oral survival of motor neuron 2 (*SMN2*) premRNA splicing modifier that increases and sustains the levels of functional SMN protein. Risdiplam has been approved in 100 countries worldwide.

RAINBOWFISH (NCT03779334) is an open-label, single-arm, multicentre study investigating the efficacy, safety and pharmacokinetics/pharmacodynamics (PK/PD) of risdiplam in infants with genetically diagnosed, presymptomatic spinal muscular atrophy (SMA) from birth to 6 weeks of age (at first dose), regardless of *SMN2* copy number.
The primary endpoint is the proportion of infants with two *SMN2* copies and baseline ulnar compound muscle action potential (CMAP) amplitude ≥1.5mV, who are able to sit without support for ≥5 seconds (assessed by Item 22 of the Gross Motor Scale of the Bayley Scales of Infant and Toddler Development, third edition) at Month 12. Secondary endpoints include motor milestone achievement; motor function; nutritional status; growth measures; survival and permanent ventilation; CMAP; PK/PD; the development of clinically manifested SMA; and safety monitoring.

The median age at first risdiplam dose was 25.0 days (range 16–41 days) for the 26 enrolled infants (data cut-off: 20 Feb 2023). The primary endpoint at Month 12 was met: 4/5 infants (80%) in the primary efficacy population were able to sit without support for ≥5 seconds (P<0.0001; performance criterion = 5%). Additionally, 7/8 infants (88%) with two *SMN2* copies were able to sit without support for 30 seconds at Month 12, including all infants with a CMAP amplitude <1.5 (n=3).

At Month 12, 24/26 infants (92%) were able to sit without support, and many were able to stand (21/26 [81%]) and walk (16/26 [62%]), as assessed by the Hammersmith Infant Neuromuscular Examination, Module 2. All infants were able to feed exclusively by mouth and had normal speech development. Additionally, all infants showed cognitive skills typical of normal child development. One infant met the criteria for development of clinically manifested SMA at Month 12.
All infants were alive without permanent ventilation after 12 months of treatment, and no adverse events (AEs) led to withdrawal or treatment discontinuation. Reported AEs were more reflective of the age of the infants rather than the underlying SMA. Most AEs were not considered to be treatment related and resolved over time.

RAINBOWFISH is ongoing globally to provide additional data on the efficacy and safety of risdiplam in infants with presymptomatic SMA.
Muscles in SMA

O21

Effect of apitegromab on motor function and patient-reported outcomes at 36 months in patients aged 2–21 years with spinal muscular atrophy

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Neuronal degeneration and muscle atrophy contribute to functional decline in spinal muscular atrophy (SMA). Apitegromab, an investigational, fully human monoclonal antibody, inhibits the pro- and latent forms of myostatin, directly targeting muscle atrophy associated with SMA.

TOPAZ (NCT03921528) is an ongoing multicenter, phase 2 study to evaluate the safety and efficacy of apitegromab in patients aged 2–21 years with Type 2 and Type 3 SMA. The study consisted of a 52-week treatment period where patients received either a 2- or a 20-mg/kg apitegromab dose, either alone (N=11) or in addition to their background nusinersen therapy (N=47). Patients who completed the treatment period could enroll in up to 3 extension periods of 52-weeks' duration for a total of 36 months where all patients continued on or were switched to receive apitegromab 20 mg/kg. The aim of the present analysis is to evaluate the effects of apitegromab in 35 nonambulatory patients over 36 months. Muscle function was measured by the Hammersmith Functional Motor Scale – Expanded (HFMSE), the Revised Upper Limb Module (RULM), and the World Health Organization (WHO) motor development milestones. Patient reported outcomes were evaluated by the Pediatric Evaluation of Disability Inventory Computer Adaptive Test (PEDI-CAT), which assessed 2 domains of function: daily activities and mobility, and the Patient-Reported Outcomes Measurement Information System (PROMIS) Fatigue questionnaire, which assessed a range of self-reported symptoms, from mild tiredness to debilitating exhaustion that could interfere with functioning. Caregivers served as proxy reporters for both assessments.

Of the 58 patients enrolled, 57 completed the primary treatment period and enrolled in the extension study. Of 57 patients enrolled in the extension period, 6 discontinued (2 withdrew consent due to COVID-19 concerns, 4 receiving apitegromab in a monotherapy arm discontinued due to lack of benefit or scheduling difficulty). Motor function in nonambulatory patients as assessed by HFMSE and RULM showed sustained improvements throughout 36 months. Sustained improvements in PEDI-CAT domain scores and perceived fatigue as measured by PROMIS were also observed over 36 months. Analysis of WHO motor milestones showed achievement of new milestones. Results on patient-/caregiver-reported outcomes were consistent with improvements in motor function assessed by HFSME and RULM. The safety profile was consistent with previous reports.

Treatment with apitegromab was associated with sustained clinical benefit for 36 months and sustained improvements in patient- and caregiver-reported outcomes of function and perceived fatigue in patients with Type 2 and Type 3 SMA. These results support further development of apitegromab in SMA.

O22

RNA-sequencing of Type II SMA paravertebral muscle after treatment reveals two distinct molecular subtypes.

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Spinal Muscular Atrophy (SMA) is traditionally considered a disease of the motor neurons, however, increasingly the systemic role of the SMN protein is being underscored. In particular, the role of the muscle as both an axis of pathology and driver of overall disease, is being appreciated. However, few datasets are available from SMA patients, especially that of Type II and III patients. With the objective to obtaining a better molecular understanding of the SMA Type II muscle and its response to treatment, we collected paravertebral muscle from SMA Type II patients treated with Nusinersen. Muscle fatigue and immobility remains a problem of Type II and Type III patients after treatment, and this dataset can allow us to determine new molecular targets for combination therapies.

Paravertebral muscle collected during spinal surgery (n=8 SMA Type II and n=7 age matched controls with no myopathies) was obtained from the Myobank-AFM (**Pitié-Salpêtrière Hospital). RNA was extracted libraries were made using the Takara** SMART-Seq v4 kit. Analysis was conducted using the nf-core rnaseq pipeline, with additional analysis for splicing variants conducted using Suppa2 and Miso.

We began by clustering SMA and control samples by their gene expression profiles and observed two groups of SMA samples with different molecular fingerprints. The first group, which we will call SMAII-A, had an overall transcriptional landscape that was like that of their age matched controls (5/8 samples). The second, which we will call SMAII-B (3/8 samples), had a transcriptional profile that was highly different from the controls. After differential expression and pathway analysis on the SMAII-B we observed that pathways related to mitochondrial metabolism and oxidative phosphorylation were downregulated. Additional pathways included the actin and calcium regulation. Among the upregulated genes, we observed several pathways related to smooth muscle contraction. Next, we characterized the SMAII-A group. While overall they did not carry the signature of the SMA-B group, a small subset of genes distinguished them from the controls. Notably, these samples had a signature of p53 activation and DNA damage. Many of these genes were also activated in the SMAII-A, showing that this signature is not exclusive to the SMAII-A group. Finally, we sought to determine if the quantity of full-length *SMN* expression might determine the clustering between the samples. However, full-length *SMN* expression was not correlated with sample grouping into SMAII-A or SMAII-B subgroups. Additional correlations with clinical variables are being tested.

This work presents, to our knowledge, the largest analysis of RNA-sequencing of Type II SMA muscles samples after treatment and provides a molecular roadmap of the state of SMA muscle after treatment. Our findings from the SMAII-B group validates previous findings about the role of mitochondrial dysfunction in SMA pathology. Work is ongoing to determine that molecular reasons – be they genetic, epigenetic, or clinical- for the heterogenous response to Nusinersen injection, and to test drug candidates to improve mitochondrial function and decrease DNA damage in skeletal muscle.

O23

ClC-1 inhibitor compound improves neuromuscular transmission and enhances skeletal muscle function in pre-clinical animal models of neuromuscular dysfunction

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ClC-1 is a Cl^- ion channel specifically expressed in skeletal muscle cells. The channel stabilizes the resting membrane potential and dampens muscle fibre excitability and is involved in regulating muscle fiber excitability during intense exercise. Recently, it was shown that inhibition of the ClC-1 channel improves neuromuscular transmission in isolated rat muscle exposed to a neuromuscular blocking agent. This pharmacological approach mimics a neuromuscular transmission failure, suggesting that ClC-1 inhibition could be a possible mechanism to improve neuromuscular transmission in neuromuscular diseases with transmission failure. While neuromuscular transmission is reliable in healthy individuals, transmission failure causes weakness and fatigue in a range of neuromuscular diseases including spinal muscular atrophy (SMA). In the present study we investigated the effect of ClC-1 inhibition in pre-clinical models of neuromuscular dysfunctions. Two animal models were used; a pharmacological model induced in healthy rats and an SMA mouse model. Our results show that pharmacological inhibition of ClC-1 restores synaptic transmission and skeletal muscle function leading to marked improvements in muscle strength in both the pharmacological model of neuromuscular dysfunction as well as the SMA mouse model. Specifically, we found that compound muscle actions potentials and stimulated muscle force were both markedly improved when animals were dosed with a ClC-1 inhibitor, and that this translated to improved running performance and grip strength. These findings suggest ClC-1 inhibition as a potential novel approach to enhancing neuromuscular transmission, thereby leading to improved muscle function and restored mobility, in disorders where neuromuscular transmission is compromised.

O24

Taldefgrobep Alfa: Preclinical and Clinical Data Supporting the Phase 3 RESILIENT Study in Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is a progressive, debilitating genetic condition that results from a homozygous deletion or mutation in the survival motor neuron (*SMN1*) gene, which leads to diminished levels of survival motor neuron (SMN) protein, associated motor neuron loss, and muscle weakness. Despite the use of approved SMN upregulators, many patients with SMA will experience continued muscle weakness that impairs physical function and quality of life. When used in conjunction with SMN upregulators, myostatin inhibitors have shown promise in increasing muscle mass and function in murine models of SMA. Taldefgrobep alfa (BHV-2000) is a myostatin inhibitor that both targets the myostatin pathway to directly inhibit free myostatin and blocks key downstream receptor signaling. Nonclinical studies and a well-established safety profile in patients with neuromuscular disease support continued development of taldefgrobep.

To review preclinical and clinical data on taldefgrobep and provide an overview of the phase 3, global, randomized, double-blind, placebo-controlled RESILIENT trial in SMA.

Two preclinical studies of murine SMA models using SMN delta 7 mice evaluated the combination of taldefgrobep and the SMN upregulator SMN-C1 to assess outcomes related to body weight, muscle weight, and/or muscle structure and function. Additionally, safety data on taldefgrobep were compiled from analyses across phase 1 studies in healthy adults and phase 1b/2 and phase 2/3 clinical studies in participants with neuromuscular disease.

In preclinical studies, the combination of taldefgrobep with high-dose SMN-C1 improved plantar flexor muscle function (P <.05), compared to SMN-C1 alone. The combination of taldefgrobep with low-dose SMN-C1 improved gastrocnemius weight as well as contraction and/or relaxation kinetics and restored type IIa atrophic muscle fibers, compared to use of SMN-C1 alone (P <.05 for each). In clinical studies, more than 359 individuals have received taldefgrobep to date, including 179 healthy adults and 180 pediatric participants with neuromuscular disease. Across all studies, there were no taldefgrobep-related discontinuations or deaths. The most frequently reported adverse events deemed related to the study drug involved injection site and hypersensitivity/ allergic reactions, which were mostly mild.

Preclinical data suggest that taldefgrobep plus SMN upregulation offers a potential benefit in SMA. In conjunction with robust safety data from clinical studies, these preclinical results provide support for the phase 3 RESILIENT (NCT05337553) trial. RESILIENT will evaluate the efficacy and safety of taldefgrobep in ambulatory and nonambulatory participants with SMA, ages 4-21 years, who are receiving SMN-upregulating therapies, regardless of their SMA type.

Monitoring disease outcome

O25

Aconitase as a marker of early pathological state in SMA: data from spinal cord and fibroblasts

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In the last years, increasing evidence indicates that dysregulation in mitochondrial functions and dynamics affecting cellular homeostasis, can be crucial in heritable diseases such as Spinal Muscular Atrophy (SMA). In our work, we investigated the role of mitochondria in SMA both at central and peripheral level in lumbar spinal cord and mouse embryonic fibroblasts (MEFs) from SMA mouse model (*SMN2*+/+; SMNΔ7+/+; *Smn*-/-), and in human fibroblasts derived from patients. Quantifications from TEM images of the spinal cord pointed out significant subcellular alterations *i.e.* cytoplasm shrinkage, neurite degeneration, autophagic features, oedema and cristae fragmentation in SMA compared to healthy controls. *In silico* Mitochondrial Network Analysis (MiNA) in MEFs underlined an increased mitochondrial network fragmentation and a larger mitochondrial footprint, suggesting the presence of giant and swollen organelles in SMA. Moreover, mitochondrial dynamics measured by the expression of markers of fusion and fission confirmed an unbalance toward fission, suggesting the activation of mitophagy in SMA spinal cord. Indeed, from a 2DE-MALDI screening on purified spinal cord mitochondria for differentially expressed targets in SMA, we identified Aconitase, a responsive redox sensor which plays a major role in mitochondrial oxidative damage. Co-immunoprecipitation and enzymatic tests revealed that in murine and human fibroblasts from patients, Aconitase undergoes ubiquitination and loss of function during disease. Finally, since SMA pathogenesis has been associated also with autophagic dysfunctions, which correlate to changes in mitochondrial content and activity, we checked for autophagic features in human fibroblasts. Interestingly, we did not find any regulation of autophagic process, suggesting that in peripheral tissues the accumulation of mitochondrial alterations *per se* does not activate autophagy.
Altogether, our results suggest that Aconitase could represent a promising new therapeutic target for combinatorial therapy with the currently available *SMN*-based therapies and a potential peripheral and non-invasive biomarker to follow disease progression.

O26

Cold-induced weakness in adolescents and adults with spinal muscular atrophy (SMA)

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SMA is characterized by progressive muscle weakness. Patients also frequently report temporary aggravation of weakness during (severe) cold exposure, a phenomenon previously documented in patients with multifocal motor neuropathy (MMN; a rare inflammatory polyneuropathy) and Hirayama's disease. The presence of temperature-induced symptoms (i.e., weakness, numbness, tingling, pain, and overall fatigue), has never been systematically assessed in patients with SMA. We therefore aimed to investigate the frequency and severity of temperature-induced symptoms, particularly cold paresis, in adolescents and adults with SMA. Additionally, we explored potential associations between patient specific factors and the occurrence of the reported symptoms.

We conducted an observational, longitudinal cohort study in genetically confirmed adolescents and adults with SMA types 1c-4 and included a control group of age-matched healthy controls. We assessed the presence and intensity of temperature-induced symptoms using a questionnaire. All patients were treatment-naïve for *SMN*-targeting therapies. Current motor ability was classified according to their ability to walk independently or not, and motor function was assessed with the Revised Upper Limb Module (RULM) and Hammersmith Functional Motor Scale Expanded (HFMSE). We analyzed various patient factors (e.g., SMA type, current motor ability, motor function, and *SMN2* copy number) in relation to the occurrence of cold-induced symptoms.

We included 103 patients (median age 39 years, range 13-67 years) and 25 healthy controls. Patients had SMA type 1c (n=7), 2 (n=46), 3 (n=47) or 4 (n=3). Eighty percent (n=82) of patients were non-walkers, the rest of the patients were walkers.
None of the healthy controls reported cold induced symptoms. Cold-induced symptoms were present in 87% (n=90) of patients and were reported as weakness (85%, n=88), with or without additional numbness (20%, n=21), tingling (15%, n=15), pain (16%, n=16), and fatigue (29%, n=30). Cold-induced weakness was present in 100% of patients with SMA type 1c and 2. Thirty-five percent of current walkers reported cold-induced weakness. Motor function scores correlated with cold paresis in non-walkers, but not in walkers.

This study shows that cold-induced weakness is a prevalent and important symptom experienced by the majority of patients with SMA and is associated with SMA severity. Further insights on the mechanisms that underlie these temperature-induced symptoms may help to develop treatment strategies.

O27

Mass-Spectrometry-based proteomics on cerebrospinal fluid identified novel potential biomarkers for Spinal Muscular Atrophy

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The availability of disease-modifying therapies and newborn screening programs for spinal muscular atrophy (SMA) has generated an urgent need to identify reliable biomarkers to monitor disease progression, therapeutic response and classify patients according to disease severity.

Objectives of this study were to identify potential biomarkers for disease severity, and to describe changes in the proteomic profile after one year of nusinersen administration. in this multicenter retrospective longitudinal study, we employed an unsupervised mass spectrometry-based proteomic approach (LC-MS) on cerebrospinal fluid (CSF) samples collected from 61 SMA patients treated with nusinersen (SMA1 n=19, SMA2 n=19, SMA3 n=23) at baseline and after 300 days of treatment.
A machine learning classifier approach (Random Forest, RF) was applied to exploit proteins able to stratify disease severity at baseline. Bioinformatics analysis was performed to investigate Gene Ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) enriched signaling pathways of differentially expressed proteins (DEPs) after one year of treatment.

The RF algorithm identified ten putative SMA proteins able to discriminate the different SMA subtypes based on their expression at baseline.
Analysis of changes in proteomic profiles identified 147 DEPs after nusinersen treatment in SMA1, 135 in SMA2, and 289 in SMA3. Overall, Nusinersen-induced changes on proteomic profile were consistent with i) common effects observed in all SMA types (i.e. regulation of axonogenesis and complement system), and ii) disease severity-specific changes, namely regulation of glucose metabolism in SMA1, of coagulation processes in SMA2, and of complement cascade and peptidase activity in SMA3.

By analyzing a large cohort of CSF samples from SMA patients, this study has identified new potential biomarkers across disease severity, and provided new insights on biological processes perturbation after one year of nusinersen treatment.

O28

H-reflex as a sensitive biomarker of sensory-motor circuit dysfunction in SMA mice and patients

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Motor impairments in patients and mouse models of spinal muscular atrophy (SMA) are largely caused by dysfunction of sensory-motor circuits. Hallmarks of motor circuit pathology are degeneration of motor neurons (MNs), and dysfunction and loss of sensory and neuromuscular junction synapses. Sensory (Ia proprioceptive) synaptic loss occurs pre-symptomatically prior to motor neuron death, making it the earliest and most conserved disease feature across different mouse models of SMA. Importantly, restoration of proprioceptive synaptic function improves motor function in both mouse and fly models of SMA, underlying the significance of sensory synapses on motor neuron dysfunction in SMA. One clinically well-established measure for the Ia proprioceptive-motor circuit is the Hoffman reflex (H-reflex). Whether proprioceptive dysfunction occurs in SMA patients, similar to SMA animal models, has not been established. It is also unknown whether defects in proprioceptive transmission extend from proximal to distal motor circuits in SMA mice during disease progression.

Proprioceptive neurotransmission onto individual 5th lumbar segment (L5) MNs in late-stage SMNΔ7 mice were investigated by whole-cell patch-clamp recordings following stimulation of proprioceptive axons. In addition, to quantify the muscle-response (M-response) and proprioceptive-mediated-response (H-reflex) in SMNΔ7 mice, we used the *ex vivo* spinal cord-hind limb preparation and assessed the sensory-motor circuit innervating the tibialis anterior muscle. We also measured the M-response and H-reflex in control and ambulatory SMA patients. Some patients were treated with Nusinersen. Finally, immunohistochemistry and confocal imaging were used to quantify proprioceptive synapses in both murine and human *post-mortem* spinal cord tissue.

We found that impairment of proprioceptive neurotransmission occurred at late stages of disease in motor circuits innervating distal limbs, in the absence of significant loss of proprioceptive synapses and MN numbers, suggesting that impaired function of proprioceptive synapses might be a sensitive measure for disease progression. Recordings from the *ex vivo* spinal cord-hind limb assay revealed amplitude reductions of 50% in the M-response and 75% in the H-reflex of SMA mice. Daily intraperitoneal injections of SMN-C3, which is a compound similar to the FDA-approved drug Risdiplam, improved both M-response and H-reflex in SMA mice. To investigate whether proprioceptive degeneration also occurs in humans, *post-mortem* tissue from 4 controls and 6 SMA patients revealed an almost complete lack of proprioceptive synapses from MNs in SMA patients. To assess proprioceptive function, muscle recordings of 6 control, 3 naive SMA patients and 4 Nusinersen-treated SMA patients revealed that M-response was reduced by approximately 70%, the H-reflex was nearly absent, consistent with severe loss of proprioceptive synapses in SMA patients. Strikingly, SMN-restoring treatment with Nusinersen did not improve the M-response, but had a strong beneficial effect on the H-reflex in treated patients.

Our findings suggest that proprioceptive dysfunction is a sensitive measure of motor circuit and behavioral impairments in both SMA mice and ambulatory patients. The ease of access of the Hoffman reflex may therefore serve as a reliable biomarker for clinical assessment of disease progression and efficacy of SMN-restoring therapies in SMA patients.

This work was supported by grants from NINDS, NIH (R01 NS078375, R01 NS125362, R01 AA027079), SMA Foundation and Project ALS to G.Z.M.; SMA Europe to C.M.S; SMA Foundation to C.J.S.

FLASH POSTER PRESENTATIONS

SESSION 1 - THURSDAY 14 MARCH
14.15 - 14.30

P141
Pain in spinal muscular atrophy patients
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Patients diagnosed with spinal muscular atrophy (SMA) frequently experience chronic pain, increased fatigue and impaired daily living activities. This study aimed to investigate the prevalence, clinical characteristics and demographic data of chronic pain in SMA patients.

Chronic pain prevalence, clinical features, motor functions, and the characteristics of the pain felt by the patients who were followed up with a diagnosis of SMA type II and type III at Eskişehir Osmangazi University Faculty of Medicine between June 2023 and September 2023 were evaluated. About pain status parameters; Pain intensity, frequency, duration, location using a body map, and factors that aggravate and alleviate pain were reported.

A total of 13 patients were included in the study. Nine patients were being followed up with a diagnosis of SMA type II (mean age 117.3±50.2 months) and four patients were diagnosed with SMA type III (mean age 148.5±51.9 months). The prevalence of chronic pain in type II and III patients was 88.8% and 75%, respectively. Pain intensity in SMA patients was mild, but the pain usually occurred intermittently, once or a few times a month, mostly in the back and lower extremities. It was determined that stretching and posture disorders increased the pain the most during physical therapy, and the distraction strategy was most frequently used as a method of coping with pain.

Pain should always be evaluated systematically in children diagnosed with spinal muscular atrophy.

P172
Criteria for identification and accurate quantification of spinal motor neurons in healthy and disease mouse models
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Motor neuron (MN) death is the hallmark of the MN diseases spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS). Quantification of MN loss in mouse models is an important readout for disease progression and therapeutic assessment. The large variability in MN death reported by different groups, even within identical mouse models, may depend on different technical approaches to label MNs as well as investigating distinct areas of the spinal cord with differential vulnerability.

MNs of selected segments of lumbar spinal cords of BL6 mice were labelled via ventral-root backfills and afterwards immuno-stained for choline acetyltransferase (ChAT). Whole lumbar spinal cords were cleared and imaged via confocal microscopy. Furthermore, selected spinal segments of SMNΔ7, SOD1-G93A and control animals were cut at a vibratome, processed for ChAT immunoreactivity, and imaged via confocal microscopy. Additionally, spinal sections of BL6 mice were stained for ChAT, SMI-32 and Nissl or ChAT and Hb9 and imaged.

Here, we established several morphological criteria to ensure consistent quantification of MNs. First, we describe a ventral-side-up spinal cord dissection, allowing segment specific MN isolation and counting. In combination with *ex vivo* ventral-root back fills and immunohistochemistry, we conclude that ChAT and HB9 are a reliable set of markers for MN identification combined with position in ventral horn. In contrast, Nissl and SMI-32 immunoreactivity are not selective and therefore, not suitable for MN number quantification. Second, ventral-root back fills of MNs within select lumbar segments with different fluorochromes, combined with tissue clearing and ChAT immunoreactivity showed that different spinal segments contain different numbers of MNs. Third, MNs marked with HB9 in select spinal segments were counted by an automated open-source counting plug-in for FIJI ImageJ software to provide an unbiased quantification of MNs. Fourth, comparison of MNs within the lumbar enlargement of SMA mice revealed a progressive degree of MN loss from L1 to L6. Interestingly, while a severe SMA mouse model exhibits selective MN death restricted to specific spinal segments, MN loss was evident throughout the entire axis of the spinal cord in the ALS mouse model SOD1-G93A.

Our detailed procedural account demonstrates that a select set of criteria is required for the valid identification of motor neurons and their accurate quantification in normal and disease mouse models. Furthermore, our results can be used as a reference for future studies requiring accurate assessment of MN counts as part of therapeutic assessment.

Acknowledgments
This work was supported by grants from the DFG (SI 1969/3-1, SI 1969/2-1) and SMA Europe 2020 to C.M.S. and by grants from NINDS, NIH (R01 NS078375, R01 NS125362, R01 AA027079), SMA Foundation and Project ALS to G.Z.M.

P13

Longitudinal changes in compound muscle action potential (CMAP) and their association with motor function in children with infantile-onset SMA in ENDEAR/ SHINE

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*At the time of development

Compound muscle action potential (CMAP) is a validated, noninvasive, and simple to perform electrophysiological measurement providing physiologic information about motor units. To evaluate the potential of CMAP as a biomarker for therapeutic effects in spinal muscular atrophy (SMA), we examined longitudinal changes in CMAP in children with symptomatic SMA treated with nusinersen and their associations with motor function scores and the achievement of independent sitting.

Our analysis included participants with symptomatic infantile-onset SMA (symptom onset ≤6 months [mo], 2 *SMN*2 copies) who initiated nusinersen in the Phase 3, randomized, sham-controlled ENDEAR study (NCT02193074) or the open-label, long-term extension SHINE study (NCT02594124; 27 August 2019 data cut). Baseline (BL) characteristics at nusinersen initiation and longitudinal changes in peroneal and ulnar CMAP amplitudes after initiating nusinersen were analyzed. Associations between changes from BL in CMAP and Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) score at each visit were examined using Spearman correlation coefficients. Early CMAP response at Day 183 as a predictor of time to achieving independent sitting at later visits was examined using Fine-Gray subdistribution hazard models.

BL characteristics of 105 participants showed very low CMAP amplitudes and disease severity at nusinersen initiation. Mean peroneal and ulnar CMAP amplitudes were between 0.11 and 0.46 mV across three age groups with symptomatic SMA (<6 mo, ≥6 mo to <10 mo, and ≥10 mo to <23 mo). Both peroneal and ulnar CMAP amplitudes increased significantly after nusinersen initiation. The mean (SD) changes from BL for peroneal and ulnar amplitudes were 0.65 (0.75) and 0.26 (0.36) mV at Day 394, and 1.17 (1.22) and 0.49 (0.67) mV at Day 818, respectively. Increases were greater for participants who were younger vs. older at first nusinersen dose. Correlations between changes in CMAP and CHOP INTEND at Day 394 were 0.28 and 0.45 for peroneal and ulnar amplitude, respectively; the corresponding correlations were 0.46 and 0.42 at Day 818 (all $p<0.05$). Participants with increased CMAP at Day 183 were more likely to achieve independent sitting at later visits. Those with ≥0.5 mV increases in peroneal and ulnar CMAP at Day 183 had 1.9-fold (95% CI, 0.9–3.9) and 4.6-fold (95% CI, 1.3–16.7) increased probability of achieving independent sitting during the study, respectively.

Despite very low CMAP amplitudes at BL, children with symptomatic infantile-onset SMA treated with nusinersen showed continual increases in both peroneal and ulnar CMAP. Longitudinal changes in CMAP correspond with the observed improvement in motor function and are predictive of achieving independent sitting at later visits. CMAP may be a potential biomarker for therapeutic effects in infantile-onset SMA. Study Support: Biogen.

P50

Dopaminergic system role in a *C. elegans* model of SMA

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SMA has been considered a motor neuron specific disorder, but this selectivity is in contrast with the fact that *Smn1* is a ubiquitous gene with housekeeping functions. Indeed, recently SMA has been re-defined as a multi-system disorder. In iPSCs-derived neurons from SMA patients, in mice and in *C. elegans* models of SMA, genes of the dopaminergic pathway resulted to be dysregulated at the transcriptional and post-transcriptional level.

We took advantage of multiple *C. elegans* SMA models mutated in *smn-1,* the *C. elegans* ortholog of *Smn1,* to investigate by HPLC, formaldehyde induced fluorescence (FIF), behavioral tests and genetics, the unexplored connection between SMA and the dopaminergic (DA) system *in vivo*.

We performed DA quantification in total extracts and *in vivo* (by detecting with FIF the DA related-fluorescence in dopaminergic neurons), and revealed a reduction in total and in intracellular dopamine. A DA-related behavior in *C. elegans* SMA models was found impaired, suggesting that the reduction of dopamine causes an alteration in the capacity to recognize the presence of food. We also confirmed the reduction in intracellular dopamine and in recognition of food in animals silenced for *smn-1* only in dopaminergic neurons, suggesting a cell-autonomous role. Since *bas-1*/AADC(*Aromatic L-amino acid decarboxylase)* expression has been found reduced in several mutant models of SMA, we overexpressed *bas-1* in dopaminergic neurons and partially rescued the behavior defect. Further, the administration of the DA precursor L-DOPA was able to partially rescue the reduction observed in intracellular DA and the behavioral defect. Interestingly, the overexpression of *bas-1* was also able to rescue a SMA-related defect, by ameliorating the locomotion impairment showed by *C. elegans* SMA models.

Taken together our results point out to a dysfunction of the dopaminergic system in a *C. elegans* model of SMA that, if confirmed in patients, may account for mood alterations observed in some SMA patients. Most importantly, our results may suggest new pharmacological combinatorial approaches to reduce the effects of *Smn1* loss in dopaminergic neurons of SMA patients. Interestingly, we also demonstrated, for the first time, the possibility to ameliorate SMA-specific locomotion defects by modulating a dopaminergic gene, like *bas-1*/AADC.

P121

A Combined Examination of Novel Rapid Bedside Plasma-SMN Analysis and Muscle Ultrasound May Help to Early Screen & Monitor Children with Spinal Muscular Atrophy in Clinical Setting

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Spinal muscular atrophy (SMA) is a genetic neuromuscular disorder characterized by progressive muscle weakness. In developing countries, delayed management is a common issue due to the difficulty in accessing genetic testing. Studies have reported the usage of muscle ultrasound (USG) and Survival Motor Neuron (SMN) protein measurement in the management of SMA patients. However, the optimal clinical utility remains unclear. This study aimed to optimize the usage of plasma-SMN analyses *and* muscle USG as means for SMA patients' screening and monitoring.

A combined study of a cross-sectional design followed by a 6-month prospective cohort design involving 49 SMA-genetically confirmed patients was performed. The plasma-SMN levels were measured using a novel in-house designed rapid SMN-meter and validated by immunoblotting using anti-SMN protein antibody and anti-alpha tubulin antibody as the references. Background scatter (echo intensity) analysis (BSC), muscle thickness, and architecture were used as USG parameters. Clinical scores were measured using standardized Hammersmith Functional Motor Scale- Expanded (HFMSE) and The Motor Function Measurement (MFM) score. Sensitivity, specificity, parameter changes, and correlation were analysed in the initial diagnosis and then calculated every 3 months.

The plasma-SMN measured using rapid SMN-meter showed high agreement with the immunoblotting result (kappa=0.82). Muscle USG which was used together with clinical score and plasma-SMN analysis had sensitivity of 93% (95%CI:90-96%) and specificity of 91%(95%CI:90-92%) in SMA screening. A *moderate c*orrelation (r=0.78) was noted between BSC and SMN level serial measurement with clinical scores during 6 months of clinical monitoring.

Using Muscle USG alone as screening tool may pose challenge. However, together with bedside rapid plasma-SMN measurement, it can become an efficient and non-invasive method for screening and monitoring patients with SMA. Optimizing its parameters further, can boost its clinical effectiveness in managing SMA, particularly in region with limited resources.

P12

FUS protein expression and distribution in the myopathology of 5q-associated spinal muscular atrophy type 3

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Spinal muscular atrophy (SMA) is a progressive, recessive neuromuscular disease characterized by significant reduction of SMN protein. This causes degeneration of lower motor neurons in the spinal cord and brainstem leading to weakness and muscle atrophy. Clinical severity is categorized in different subtypes (type 0-3), which is turn influenced by residual SMN level. While the genetic basis of SMA is well described, the tissue-specific molecular pathways underlying SMA are still not fully understood and identification of marker proteins (with pathophysiological relevance) in human vulnerable tissue such as skeletal muscle is still lacking.

To elucidate molecular markers in SMA-diseased muscle, we performed unbiased proteomics and transcriptomics on muscle biopsies derived from three SMA type 3 (walker) patients and moreover performed immunofluorescence studies on muscle biopsy specimen derived from five cases and on cultured myoblasts derived from three cases.

Combined proteomic and transcriptomic studies unraveled FUS (a DNA/RNA-binding protein and aggregation marker) as a potential molecular marker increased in SMA muscle. Results of our immunofluorescence studies showed a perimyonuclear disposition of FUS in a proportion of muscle cells compared to the immunoreactivity obtained in control samples.

Our data classify FUS as a protein involved in SMA-based myopathology and support the concept of skeletal muscle as a primary tissue target of SMA. Further studies are crucial to define the role of mis-localized FUS in SMA muscle.

SESSION 2 - FRIDAY 15 MARCH
14.15 - 14.30

P118
Oro facial strength in symptomatic type 1 SMA patients treated with nusinersen: Results from a prospective study involving 4 centers Milano - Rome - Brussels - Ghent

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Spinal muscular atrophy (SMA), a genetic neuromuscular disease caused by the lack of survival of motor neuron (SMN) protein, is characterized by muscular atrophy and respiratory and bulbar dysfunction, in particular in type 1 SMA. Swallowing function remains poorly studied, mainly because investigation tools such as Fiberoptic Endoscopic Evaluation of Swallowing (FEES) and Videofluoroscopic swallowing study (VFSS) are often not available or poorly tolerated by young children. A user friendly IOPI system measurement was recently developed to objectively evaluate orofacial strength mainly in type 2 and 3 SMA patients.
The main objective of this study is to investigate the level of impairment of muscles involved in the oral phase of swallowing (lips and tongue) in a cohort of type 1 SMA children treated with nusinersen when compared to healthy controls. Secondly, we aim to correlate lip and tongue strength with known key predictors of phenotype severity (CHOP Intend, age at first symptoms, SMN2 copy number), and with indicators of feeding-nutritional status such as the need for gastrostomy, the body mass index (BMI).

By combining the results of 4 independent centers that were actively investigating IOPI measurement in the context of a prospective study, we recruited 20 patients with a confirmed genetic diagnosis of symptomatic type 1 SMA. All patients were treated with nusinersen for a minimum of time 2,5 years and all had at least one IOPI measurement at the anatomical level of the tongue and lips at a median age of 5,4 years. The IOPI data were compared against age and gender-matched controls and published normative data where available.

Preliminary results showed that the oral pressure is clearly lower in both anatomical localizations (lip: n=12/20 patients – tongue: n=15/20 patients) when compared to typically developing children (lip – $p<0.01$ - tongue: $p<0.001$). There is a positive correlation between CHOP Intend score and muscle (lip) pressure ($r=0.723$, $p=0.012$). There was no difference between the median lip pressure between those without and with gastrostomy: (7 vs 6 kPa), whereas the median tongue pressure was higher in those not requiring gastrostomy (7.5 vs 4 kPa). Other correlations with key indicators of disease severity and feeding nutritional status are ongoing.

While patients treated with nusinersen clearly achieve prolonged survival and clear general motor progress, IOPI measurement could be performed in clearly objectify the weakness of oro facial musculature in this population.

P17
Exon7 Targeted CRISPR-Prime Editing Approaches for SMN2 Gene Editing in Spinal Muscular Atrophy

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Spinal Muscular Atrophy (SMA) is a neurodegenerative disease characterized by loss of alpha motor neurons. There are 2 genes, *SMN1* (Survival Motor Neuron 1) and *SMN2*, which are associated with SMA disease and these genes express SMN protein. The SMN2 gene expresses less than 10% of the functional SMN protein. Although the *SMN1* and *SMN2* genes are considered nearly identical, they differ from each other by only 11 nucleotides. The most important change between *SMN1* and *SMN2* is the inhibition of exon 7 participation as a result of C-T transformation at 840 nucleotides in the *SMN2* gene. This nucleotide change causes the exonic splicing enhancer, which operates on exon 7, to be converted into a silencer. For this reason, the elements involved in the splicing phase cannot bind to the binding site and the production of SMN protein that has lost its function, which does not contain exon 7 at a rate of 80-95%, takes place. CRISPR technology, which adds a new dimension to genetic engineering and gene therapies, allows the treatment of many genetic diseases. In terms of SMA, some previous studies in the literature prove that it is possible to treat SMA with the CRISPR strategy. CRISPR-Prime Editing (PE) technology is a next-generation gene editing approach that precisely enables a variety of genomic modifications without the need for double-strand breaks or donor DNA sequences. CRISPR Prime Editing-mediated gene editing for SMA was reported for the first time in our study (Odabaş et al., BioRxiv, 2022). The c.840 T-C transition and c.859 G-C transformations in the *SMN2* gene and the simultaneous correction of these point mutations with a single pegRNA will be examined for the first time in this project. We show that CRISPR-PE systems can increase *SMN2* gene activity and SMN protein expression by regulating c.840 T-C transition and c.859 G-C transformations, enabling exon 7 participation. We demonstrated in our preliminary work as a proof-of-concept the efficiency and stability of the Prime Editing method of modifications in *SMN2* genes investigated in SMN-low Jurkat cells, primary SMA type I, and type II fibroblast cells.

P135

Towards the identification of biomarkers of disease progression and response to treatment in spinal muscular atrophy

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Survival motor neuron (SMN) 1 gene, whose loss or mutations cause SMA, has ubiquitous expression in the organism, where it critically regulates several developmental and housekeeping cellular pathways, like RNA metabolism and biogenesis of microRNAs (miRNAs). MiRNAs are key gene expression modulators, whose dysregulation contributes to neuromuscular diseases; they are stable in body fluids and reflect distinct pathophysiological states, acting as promising biomarkers (BMs). Growing evidence suggests that
intrinsic skeletal muscle defects contribute to SMA pathology and we already demonstrated that the expression levels of circulating muscle-specific microRNAs (myomiRs), miR-133a, -133b, miR-206 and miR-1, decrease under nusinersen therapy in pediatric SMA. However, their potential as BMs for clinical use in adult SMA patients has not been investigated yet.

To analyze the expression profile of myomiRs in serum of adult SMA patients before and during nusinersen treatment to investigate their role as potential non-invasive prognostic and pharmacodynamic biomarkers.

Serum was collected from adult (>18y/old) SMA patients. Total RNA was isolated from serum using miRNeasy Advanced Serum kits (Qiagen). Expression levels of myomiRs at baseline, and after 6 and 14 months of nusinersen treatment, and in age-matched healthy controls, were assessed by RT-qPCR using Taqman microRNA Reverse Transcription kit and specific primers. Motor function assessment was performed by the Hammersmith Functional Motor Scale Expanded (HFMSE). Data analysis was performed by Mann Whitney test and Spearman's correlation analysis.

Two type-2 and 14 type-3 adult SMA patients (8 female; 2 non sitters, 2 sitter and 10 walkers) were enrolled. We detected an upregulation of miR-206, -133a and -133b before treatment in serum of adult SMA patients compared to controls ($p<0.001$; $p<0.005$ and $p<0.05$, respectively). A common pattern of reduced
expression till normalization of miR-206, -133a, -133b was evident in SMA patients upon 14 months of nusinersen administration, till normalization. Of note, baseline levels of miR-133a positively correlated with motor function at baseline and after treatment.

This investigation supports the role of serum myomiRs as non-invasive BMs to monitor disease progression and therapeutic response also in adult SMA, laying the groundwork to individualize patient management in the clinical practice. Particularly, in the clinical setting, miR-133a could help identify those patients most likely to demonstrate a meaningful response to nusinersen therapy.

P64

Unravelling the role of GABA signalling and metabolism (dys)regulation in Spinal Muscular Atrophy: Results from SMAΔ7 mice cortex

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Spinal muscular atrophy (SMA) is a genetic neuromuscular disease affecting children and young adults, characterized by motor neuron (MN) impairment and skeletal muscle atrophy. However, we also observed a selective degeneration of motor cortex (CRTX) layer V pyramidal neurons in SMAΔ7 mice (a severe SMA murine model), compared to WT controls, suggesting that its pathogenesis is likely more complex than previously anticipated. To date, although the monogenic causes of the disease are well known, many other aspects are still unclear, and the available therapies still show many limits. Intriguingly, neuroprotective effects of GABA-targeting drugs were reported in SMA, suggesting a possible dysregulation of GABA (the main inhibitory neurotransmitter of the central nervous system) and inhibitory interneuron (IN) pathways at cortical level, as a common aetiology shared with other neuronal diseases.

We have strong preliminary results showing perturbation of GABA metabolism and Parvalbumin (PV) IN functions in SMAΔ7 mice sensorimotor (SM) CRTX, in the late disease stage (postnatal day 12), in comparison with WT. By immunofluorescence (IF) analysis, we observed a significant reduction of GABAergic signal (-57%, $p<0.01$) and reduced density of GABA+-cells (-25%, $p<0.01$) in SMAΔ7 SM CRTX, along with an impaired distribution and reduction of GAD65 and GAD67 (GABA synthesis enzymes) signal (-60% and -65%, respectively, $p<0.05$) and GAD67+-cells (-20%, $p<0.05$), underlying neurotransmitter synthesis defects. Moreover, PV INs were found significantly reduced in number (-34%, $p<0.05$) and morphologically altered, suggesting possible failure in their inhibitory functions. Immunoblotting further confirmed a reduction in GAD65/67 protein levels in the SMAΔ7 SM CRTX (-25%, $p<0.05$). Furthermore, IF analyses in SMAΔ7 motor CRTX showed a reduction of GABAergic synapses contacting-neurons in layers II/III (-28%, $p<0.01$) and V (-38%, $p<0.001$), suggesting loss of GABAergic contribution to cortical excitatory/inhibitory homeostasis.

Overall, these results show for the first time GABAergic dysregulations in the SM CRTX of SMA mice, possibly contributing to the onset of the disease. Further studies aimed at fully understanding and pharmacologically rescuing GABA pathways will pave the way for new SMA treatments.

P100

Electrophysiological assessment of motor unit patterns of the median nerve in adolescents and adults with spinal muscular atrophy (SMA)

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* Equally contributed to this work.

SMA affects multiple parts of the motor unit. Electrophysiological techniques might offer insights into disease severity and motor unit reserve, and potentially serve as therapeutic biomarkers. We investigated motor unit patterns and their correlation with clinical factors in adolescents and adults encompassing a large part of the SMA spectrum, by using an electrophysiological protocol including compound muscle action potential (CMAP) scan and motor unit number estimation (MUNE).

We conducted a national, cross-sectional cohort study of Dutch adolescents and adults with genetically confirmed SMA types 1c-4 and in a healthy control group. None of the patients used *SMN*-augmenting therapies. We performed the CMAP scan on the median nerve, and derived $CMAP_{max}$, MUNE absolute mean motor unit (MU) size, and largest unit size (in mV), as well as the relative contributions of large and small MUs to the total pool (as % of $CMAP_{max}$). We explored associations of these CMAP scan markers with clinical characteristics, including SMA type, *SMN2* copy number, age, disease duration, motor function scores (HFMSE, RULM, and ATEND), and current motor ability (categorized as unsupported sitters, supported sitters, and walkers).

We included 104 SMA patients (median age 39, range 13-67) and 65 controls (median age 58, range 13-79). SMA types included 1c (n=7), 2a (n=29), 2b (n=17), 3a (n=32), and 3b/4 (n=19). Forty-seven percent were unsupported sitters, 33% supported sitters, and 20% walkers.
CMAP scan markers differed between patients and controls. In healthy controls, age had an inverse correlation with MUNE and positive correlation with the mean MU size (as % of $CMAP_{max}$).
Within SMA, age did not correlate with $CMAP_{max}$ in any type. Disease duration negatively correlated with $CMAP_{max}$ in patients with SMA type 3b/4. Both age and disease duration negatively correlated with MUNE and positively correlated with mean MU size (as % of $CMAP_{max}$) in patients with SMA type 1c. $CMAP_{max}$ and MUNE correlated with disease severity, as reflected by SMA type, *SMN2* copy number, motor function scores, and current motor ability. Absolute MU sizes (mean MU and largest unit size) did not correlate with disease severity. However, the relative contribution of small as well as large units differed with disease severity. In more severely affected patients, larger MUs constituted a higher percentage of the total pool, also reflected by a higher mean MU size (as % of $CMAP_{max}$).

This study explored motor unit patterns in a wide spectrum of patients with SMA and longstanding disease. We observed differences in electrophysiologic markers between disease severities, in particular motor unit quantity and the composition of large and small units and their relative contributions to the total pool. Although these findings do not conclusively establish a correlation with age or disease duration, they show promise as a biomarker in other patients than infants.

P47

Network biology-based analysis of SMA: Identification of disease relevant protein targets and altered signaling in severe and mild SMA mice

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Strategies for increasing SMN expression levels have been a ground-breaking step for the treatment of Spinal Muscular Atrophy (SMA). However, SMN is involved in several molecular and cellular mechanisms and the identification of disease-relevant processes is challenging. This complexity of SMA results in the need for a network-biology analysis approach. In this project, we aim to (1) identify expressional and phosphorylation changes in spinal cord samples at disease onset in different SMA mouse models (Taiwanese, $Smn^{2B/-}$) and (2) analyze signaling alterations and kinase phosphosite associations of significantly dysregulated proteins. To combine the current knowledge about SMN and the mechanisms altered in SMA, network analysis was used. We additionally used SMN-interactome data sets to mechanistically elucidate the molecular link to SMN in the network of dysregulated targets. This network highlights hubs and bottleneck molecules at critical positions.

To better understand the central pathways of SMA, we performed quantitative (phospho) proteomic analyses of L1-5 spinal cord from the severe Taiwanese and the mild $Smn^{2B/-}$ mouse model at a presymptomatic stage (P3 and P12-13, respectively).

We identified 330 and 220, respectively, dysregulated proteins with an overlap of 32 proteins and several upstream regulators. Those dysregulated proteins and regulators include already described proteins in SMA, as e.g., Small nuclear ribonucleoproteins, neurofilament proteins and mTOR or MAPT. Both data sets include targets involved in splicing, cytoskeletal proteins, translation, and DNA replication (severe model only). Therefore, the data reflect SMA at different disease stages. Phosphoproteome analyses of both models revealed changes in signaling pathways and kinase families associated with dysregulated phospho-sites.

This study combines different molecular levels and methods to describe SMA on a systems level and enables the interpretation of single protein changes in the disease context.

POSTER SESSION 1

THURSDAY 14 MARCH

16.00 - 17.30

Developmental aspects of SMA

P1
Cell-autonomous disease mechanisms in spinal muscular atrophy with respiratory distress type 1 (SMARD1)
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Muscle atrophy and diaphragmatic palsy are the clinical features of SMARD1, which is well represented in the neuromuscular degeneration (*Nmd*^{2J}) mouse. In both humans and mice, mutations in the IGHMBP2 gene lead to motoneuron loss. IGHMBP2 is a ribosome-associated ATPase/helicase involved in ribosomal and translational processes. However, the disease mechanisms at the cellular level leading to SMARD1 are far from being understood. We have shown that IGF1 treatment improves motor function with reduced fiber degeneration in the gastrocnemius muscle and diaphragm, but has no beneficial effect on motor neuron survival. An analysis of primary Ighmbp2-deficient motor neurons shows only minor but obvious morphological changes, such as an increase in axonal branches. RNA sequencing revealed few changes in the transcriptome. Similarly, no global changes in protein synthesis are detectable, except for a reduced ß-actin protein level at the growth cone. To better elucidate the cell autonomous mechanisms, we performed a proteomic analysis of primary cultured Ighmbp2-deficient motoneurons.

For proteomic analysis, Ighmbp2-deficient and control motoneurons from E12.5 mouse embryos were cultured for 7 days. On day 7, cells were lysed and 10 µg protein lysate was processed for proteomic analysis. Western blot and immunohistochemical staining were performed to verify each target. For Western blot analysis, primary motor neurons were cultured for 7 days and 10 µg of protein extract was loaded onto a 6% gel. Western blots were analyzed using an imager from Intas and ImageJ software. For immunohistochemical analysis 7-day-old motoneurons were fixed with 4% PFA and treated with the appropriate antibodies. Images were captured with an Olympus microscope and analyzed with ImageJ software.

Proteomic analysis of primary cultured Ighmbp2-deficient motoneurons revealed a small number of up- and down-regulated targets. We focused our analysis on two of these targets. One target is the extracellular matrix protein tenascin C. The second target is the epidermal growth factor receptor (EGFR).
Both molecules are downregulated in cultured *Nmd*^{2J} mouse motor neurons. We verified the downregulation of both targets by Western blot and immunohistochemical analysis.

TnC is an extracellular matrix protein associated with EGF/EGFR signaling with an important function in cellular integrity during embryonic development. To elucidate whether TnC and EGFR down-regulation in Ighmbp2 deficient mice leads to impaired embryonic development, specifically during differentiation and maturation of motoneurons, we will investigate TnC distribution and EGF/EGFR signaling between E11.5 and P5 in *Nmd*^{2J} and control mice.

P2
Bone-intrinsic dysregulations of the mandible are associated with altered collagen synthesis in a severely affected SMA mouse model
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SMA, characterized by reduced regenerative capacity, results in a dysregulated network of various factors. To fully understand the factors needed to improve therapeutic strategies, the causes of dysregulation need to be analyzed in more detail. In previous studies, we have shown that pre-symptomatic P1 severely affected "Taiwanese" SMA mice have smaller vertebral bodies and shortened femora. They also exhibit transcriptional changes leading to dysregulated collagen formation and remodelling. Both processes play an important role in cartilage formation and bone development. Our findings suggests that SMA affects collagen synthesis in different organs. A dysregulation of collagen IV was also shown in the kidney at pre-symptomatic P3. Furthermore, growth-related craniofacial dysmorphologies as well as reduced mandibular mobility and restricted mouth opening can be diagnosed clinically in SMA patients as well as phenotypically in the severely affected mouse model. In the present study, potential transcriptional dysregulations of pre-symptomatic mandible including tooth germs and temporomandibular joint using Nanopore long-read sequencing were investigated.

"Taiwanese" SMA mice were decapitated on pre-symptomatic P3. The whole mandible was prepared and used for transcriptomic analyses by Nanopore sequencing. Briefly, data were base called using *MinKnow*, mapped to the mouse reference genome using *Minimap2* and further analyzed by *DeSeq2*. The pipeline detected dysregulated transcripts, which were subsequently analyzed by *STRING* network analysis to identify relevant nodes of putative pathomechanisms. Selected network nodes were analyzed by qRT-PCR and Western blot.

Bone transcript dysregulations were detected in the mandible of severe SMA mice at P3. Network analysis identified an independent collagen cluster of four interacting nodes. One node of this collagen cluster is *Collagen Type I alpha 1 chain*. This protein is involved in skeletal system development, ossification, osteoblast differentiation and intramembranous ossification. The identification of this collagen cluster provided more information about its importance in bone development and ossification of the mandible.

The study identified a large number of dysregulated transcripts as relevant nodes of putative pathogenic processes at a pre-symptomatic stage of SMA mice. Main result was that collagens were dysregulated in the mandible, corroborating the results of previous studies showing a general dysregulation of collagens in SMA. However, the analysis of differentially regulated transcripts provided information about dysregulated networks at a higher level of regulation. Further studies are necessary to investigate the role of craniofacial alterations especially of the mandible and the temporomandibular joint in this disease with the aim enabling dentists to develop treatment strategies to reduce or ideally prevent mandibular malformations.

P3

Investigating the developmental expression of SMN in mouse brain

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Spinal muscular atrophy (SMA) type 1 (SMA1) is the most common and severe form of SMA with symptoms onsets within the first six months of life. The extended survival of treated SMA1 children is enabling us to appreciate neurodevelopmental phenotypes that were not seen before, as untreated patients did not survive beyond the age of two years. Recent small cohort studies show that treated SMA1 patients may have delayed cognitive and communication development, and this is also reported in rare patients with two *SMN2* copies treated pre-symptomatically. These preliminary observations unveil that brain-related comorbidities can be present in a proportion of SMA patients, suggesting a possible role of SMN in brain development.

The aim of this project is to elucidate the effect of SMN deficiency on brain development, both at the structural and molecular level. To do this, we use the Taiwanese severe SMA mouse model, where mice are treated upon birth with either of two different *SMN2* splicing modifiers, an ASO (treated once at birth) or a small molecule (treated daily), to restore *SMN2* exon 7 splicing. Our aim is to investigate the expression of *SMN* transcripts and protein levels in brain tissue, comparing to wildtype (WT) and untreated SMA1 mice, as well as investigating how these levels change with age. Untreated SMA type 3-like (mild phenotype) with four copies of human *SMN2* are also used. The role of the SMN complex in the spliceosomes (e.g. small nuclear RNPs (snRNPs)) will also be investigated.

Preliminary data show that levels of full-length (FL) SMN and Δ7 transcripts and SMN protein in brain decrease with age in WT, SMA1 and SMA3 mice, consistently with data in humans. SMA1 mice treated with either of the two *SMN2* splicing modifier have increased levels of SMN protein in cortex and cerebellum, compared to untreated SMA1 mice of the same age.

Brain tissue is currently being collected at more frequent time intervals to better define the temporal changes of protein and transcript levels in the brain of the mouse model of SMA treated with SMN-enhancing therapies. Spinal cord will also be collected to investigate temporal changes in SMN protein and transcripts. We will also investigate levels of different proteins which are part of the spliceosomal complex that function in pre-mRNA splicing, to clarify how this affects temporal changes of *SMN* splicing.

P4

Longitudinal developmental profile of newborns and toddlers treated for spinal muscular atrophy – Follow-up

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The cognitive and developmental aspect of neurodevelopmental pathologies is not yet sufficiently represented in the literature. This is also the case regarding the SMA. The aim of this study is to highlight developmental trajectory of treated patients with spinal muscular atrophy type 1 (SMA1) and presymptomatic patients using Bayley Scales of Infant and Toddler™ – Third Edition (BSID-III) and Fourth Edition (BSID-IV).

This monocentric prospective observational study included, 10 patients with SMA1 and 5 presymptomatic patients with SMA, all treated with an approved drug after and before the onset of symptoms, respectively. 4 patients were added to the longitudinal group counting now 19 patients. They are evaluated every 3-4 months from September 2018 until now.

At each time point, all patients treated presymptomatically scored above those treated post-symptomatically on the motor scale. The cognitive scores of four or the five patients treated presymptomatically were average; one patient was in the low average range. On the communicative scale, three of the five presymptomatically treated patients scored average, one scored low average, one abnormal. In the ten post-symptomatically treated patients, six scored either in the low average or the abnormal range on the cognitive scale, and seven scored below average in the communicative composite score. The rest of the data is currently being analyzed.

Patients with SMA1 treated before symptom onset had better motor outcomes than patients treated post-symptomatically, in line with previous data. A significant proportion of patients treated post-symptomatically scored below average on cognitive and communicative scales. Our study indicates that intellectual development should be considered as an important outcome in patients with SMA1 treated with recently approved drugs. Cognitive and communicative evaluations should be performed as part of standard of care, and guidance should be provided to parents for optimal stimulation, particularly regarding language development.

P5

Single cell RNA sequencing identifies relevant role of fibroadipogenic precursor cells in the pathogenesis of spinal muscle atrophy

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Loss of motor neurons as happens in spinal muscle atrophy (SMA) is associated to progressive atrophy and loss of muscle fibers and their replacement by fibrotic and fat tissue. The molecular pathways driving atrophy of muscle fibers are starting to be known, although there is still not too much information about the changes in the gene expression profile of muscle resident cells in skeletal muscles of SMA patients.

We performed single cell RNA sequencing to cells isolated from muscle samples of three patients with SMA type III produced by mutations in the SMN-I gene and from three age matched control samples. We applied bioinformatic analysis to identify the cell types isolated but also the genes and molecular pathways that were upregulated in cells isolated from SMA patients compared to controls.

Gene expression analysis identified two main population of cells: myoblasts and fibroadipogenic precursor cells (FAP) which were the majoritary cell types. There were 9 clusters in total, one of them representing myoblasts, and 8 representing FAPs. After reviewing the gene expression we could allocate the following identities to the FAPs: proliferating FAPs characterized by expressing genes involved in cell division, fibroblast like cells characterized by expressing components of the extracellular matrix, regulatory FAPs releasing growth factors involved in regulation of cell division and proinflammatory FAPs expressing genes coding for cytokines and inflammatory molecules. Fibroblasts and inflammatory FAPs were more abundant in the SMA samples than in controls. SMA FAPs expresssed higher levels of genes involved in extracellular matrix expansion, vascular development and very interestingly axonogenesis. SMA FAPs expressed high levels of genes such as RELN, PLXND1 or NTN4 which are involved in neurogenesis and/or axon migration suggesting that FAPs contribute to the reinnervation process in SMA patients.

FAPs seems to have a pivotal role in the pathogenesis of SMA as they participate in the expansion of fibrotic and fat tissue observed in these patients, but they can also be relevant supporting axon growth and migration on the motor terminal toward muscle fibers.

P6

Cognitive function exploration of children with spinal muscular atrophy

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In last few years, pharmaceutical studies have led to major step forward in Spinal Muscular Atrophy treatment. Before that, type I and II were more severe, even lethal. Care and support were so important than this left no place for investigating the cognitive profile of these children, even though a large proportion of the care staff highlighted the fact that SMA children look to be cognitively more advanced than average children without pathology. The hypotheses behind these observations can be of 3 different types: social, genetic or compensation due to motor limitations. Therefore, at this time, there is not enough studies carried out in the literature research to take position regarding these observations.

Henceforth, the aim of this study is to explore the cognitive function of several children with SMA (types I, II and III), aged 4 to 13, with IQ testings (WPPSI-IV and WISC-V), fluid reasoning (Raven Matrix), executive functions (Day and night test, K-ABC-II story to complete) language abilities (E.c.o.s.s.e, EVIP-A), and social cognition (Emotion recognition test, Social Resolution Test, Facial expression matching and emotional attribution task, False step test and Nepsy-II theory of mind test). These testings were conduct on a sample of 10 patients, started in february 2019.

First results of this study could not objectify the care staff's observations. The main finding is an important heterogeneity, intrapersonal as well as interpersonal. As a matter of fact, some IQ profile were upper the average, others just below the average, with most of them having heterogenous results in different domains, for which the mean is unrepresentative of the child's true abilities. Language abilities and social cognition highlighted similar results. However, we can at least point to the fact that these results are not in favor of a genetic cause to explain these observations. Therefore, sociocultural and compensation are still two good hypothesis that can be investigated.

Nevertheless, as SMA is a rare disease, the sample of participants is not big enough to conclude in a significant relation between SMA and witness. Moreover, the study should be reconducted several times to reveal the stability of results over time and with a larger sample.

P7

Development, vulnerability and recovery of intramuscular motor axons in SMA

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Although treatments for SMA have dramatically changed the prognosis for patients, the clinical response remains variable, and many patients still experience deficits in motor function. To understand what is impeding recovery it is critical to determine the capacity for repair in the neuromuscular system, specifically if motor axons remain viable postnatally and can be targeted to compensate for damage accrued prior to treatment. Axonal development is impaired postnatally in SMA, with large numbers of small unmyelinated axons in the ventral roots of type I SMA patients and in mouse models. These unmyelinated axons are at risk of rapid degeneration. Here we have undertaken a detailed analysis of intramuscular motor axon development and vulnerability in a range of muscles. We have further investigated whether motor axons which display defects in development retain the capacity to be rescued following a postnatal induction in Smn levels.

To find out when intramuscular axons are lost in SMA and if this is consistent across muscles with differing vulnerability, we undertook a time course of axonal loss in a range of cranial muscles in the delta 7 mouse model of SMA. This revealed an early and significant loss of motor axons, occurring between P1 and P3. By P3 approximately 50% of axons had been lost from both vulnerable and moderately affected muscles. In addition, axon maturation and myelination defects were observed in intramuscular motor axons which were akin to those reported in the ventral roots, and in SMA patients. To ascertain whether developmentally immature and vulnerable motor axons retain postnatal viability, we administered either SMN inducing ASO or AAV9-Smn to delta 7 mice at P2 and profiled the impact that this had upon motor unit recovery at P12. In the moderately affected auricularis auris longus (AAL) muscle, axon numbers and denervation levels were similar to those in control littermates. However, in more vulnerable muscles there was a decrease in the number of intramuscular axons and increased incidences of vacant endplates. The profound loss of axons in vulnerable muscles gave rise to an increase in motor unit size and suggests that the few remaining axons are sprouting to compensate for the loss of their neighbours.

The lack of complete recovery following treatments indicates that Smn-dependent therapies are not reaching the full capacity for repair and gives us a window in which to intervene to improve outcomes. The recovery of intramuscular axon numbers in the AAL muscle, even after the onset of axonal loss in this muscle, suggests that axons are not irretrievably lost and remain viable postnatally. This work suggests that developmentally immature axons can be rescued following a postnatal increase in Smn levels and offers the possibility for treatments to protect motor neurons and enhance regeneration working in synergy with Smn inducing compounds to support the neuromuscular system.

P8

Developmental aspects of human samples affected by SMA

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Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder mainly characterized by degeneration and loss of function of alpha motor neurons (MN) in the anterior horn of the spinal cord. Several studies indicate the involvement of additional peripheral organs in SMA, particularly associated with severe disease. Due to SMN being ubiquitously expressed, together with reported abnormalities of peripheral organs observed in SMA mouse models, there might be indication of human peripheral organs also being a target for pathology at both pre- and postnatal stages.
Here we performed histological and morphometric analysis on organ tissue of human fetuses and infants with SMA.

Our aim is to uncover possible pathology that might occur during development in human organs not directly associated with SMA

Tissues were selected in post-abortion or post-mortem samples and fixed in 10% buffered formalin for subsequent staining and immunohistochemistry. Tissues include Spleen, Thymus and Kidney.

Morphology assessment by H&E-stained tissue as a Gold-Standard of pre- and postnatal SMA samples revealed several possible indications of abnormalities in both pre- and postnatal samples of peripheral organs. Respective to each organ more specific antibody-staining was carried out to further assess expression of different specific proteins such as CD20 and CD3 of B and T lineage lymphomas respectively in spleen and thymus for possible lymphoid concentration pathology.
For the kidney CD10, a cell surface metalloproteinase localized to the proximal nephron, was stained to investigate possible abnormalities in nephron development, CD163 for anti-inflammatory macrophages and von Kossa staining to investigate possible medullary calcifications along collecting ducts.

In this limited study for the first time, we describe a systematic analysis of peripheral organs in SMA with findings of possible evidence of SMA-pathology during development supporting the idea of SMA being a multi-organ disease.

P9

CUIDAME: Descriptive data on neurocognition status of SMA1 individuals of the Spanish longitudinal project to collect data on patients with spinal muscular atrophy

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Natural history of SMA type 1 has dramatically changed due to improvement in treatment and better disease management. With the increasing number of long-term survivors of SMA1 worldwide, children show new phenotypes and some patients with SMA1 may show cognitive impairment.
CuidAME Project is collecting Longitudinal Clinical Data of Spanish SMA patients to generate Real-World data, using the SMArtcare platform. Retrospective and prospective data of SMA patients is collected in 30 sites. Patients are followed for 5 years, with a visit entered every 8 months, regardless of their treatment regimen. The data is collected during routine clinical visits and includes the main characteristics of the onset and evolution of the disease, genetic diagnosis, treatment and motor function assessments.

In this analysis we describe data on neurodevelopmental characteristics for the Spanish SMA1 cohort using a questionnaire sent to CUIDAME centres collecting retrospective data.
Eight centres followed-up of 65 individuals with SMA1, 18 of whom presented neurodevelopmental problems. The current age of the affected population was between 18 months and 6 years, all had 2 copies of SMN2 and were treated with a disease modifying therapy. Absent or severely delayed speech was the most prominent problem identified (n=12), but also communication/ social interaction difficulties (n=5) and features of ASD (n=3). Almost 50% of the population with problems had a formal neurocognitive assessment.

The results suggest that cognition, speech and language development could be affected in SMA type 1 patients. A better understanding of the neurodevelopmental function by performing formal neurocognitive assessments is crucial. To this end, a standardised assessment protocol for children with SMA1 with suspected impairments could be useful.

P10

Profiling the motor neuron translatome and transcriptome unique to the early symptomatic stage of the Taiwanese SMA mouse model

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Tight control of gene expression is key to the correct development and maintenance of cells, with delicate regulation required at both the transcriptional and translational level. With the well-known need of the SMN protein for correct spliceosome and snRNP assembly, Spinal Muscular Atrophy has been described as a disease of splicing dysregulation. However, recent studies have also highlighted the role of SMN in ribosome functions, and early disruption in translational control of mRNA transcripts is observed in SMA. Defects in both processes should therefore be considered as contributing to the pathology of SMA. The most important genes affected downstream of these defects represent excellent targets for developing non-SMN-dependent therapies and to generate new insight into mechanisms of motor neuron degeneration.

We utilised Translating Ribosome Affinity Purification followed by RNA sequencing (TRAP-seq) to isolate motor neuron-specific mRNA transcripts actively translated by ribosomes at the point of sampling. We performed TRAP-seq on the Taiwanese mouse model and healthy littermate controls, at early (P5) and late (P9) symptomatic time points. As well as motor neuron translatome datasets, we also performed RNAseq on all transcripts isolated from ventral spinal cord tissue, generating transcriptome data. These datasets thus provide cell-specific changes to gene expression *in vivo* during SMA pathology progression.

Comparative analysis of the differentially expressed genes (DEGs) between SMA and control data revealed many genes dysregulated only in the TRAP-seq translatome, suggesting a large contribution of translation-mediated dysfunction in SMA, independent of transcriptional changes. Focusing on the early symptomatic P5 time point, our data also showed that translational disruption affects a larger number of genes than in the transcriptome data, and the majority of translatome DEGs are unique to the early symptomatic stage, and do not persist in the late symptomatic stage. This demonstrates that some of the earliest DEGs contributing to neurodegeneration in SMA are compensated for at the end-stage, while other pathways are only activated or suppressed when motor neuron death is already widespread. By clustering analysis, we identified processes already known to be affected in SMA, such as metabolism and mitochondria, but also embryonic morphogenesis and extracellular matrix remodelling.

Profiling the translational changes at the earliest stages of SMA offers novel avenues into greater mechanistic insight into motor neuron degeneration, with DEGs that are unaffected at the wider transcriptional level. These translatome-specific pathways demonstrate that translational dysfunction is a large contributor to early SMA pathology, and these early changes in the motor neuron deserve further focus for potential new therapy development.

P11

Challenge in diagnosing Spinal Muscular Atrophy with childhood onset.

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Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular neurodegenerative disorder characterized by progressive lower motor neuron degeneration due to insufficient levels of the Survival Motor Neuron (SMN) protein, resulting from homozygous deletion or loss-of-function mutations within the SMN1 gene. Muscular weakness, areflexia, and fasciculations are hallmarks of SMA.

A 26-year-old man came to our attention due to progressive hypotrophy and weakness in his lower limbs. He had a family history of undetermined ataxia, possibly related to a toxic-metabolic cause. In his neurological medical history, the patient reported experiencing hyposthenia in his lower limbs with marked atrophy primarily in the proximal muscles bilaterally since the age of 12. There were no sensory disturbances. Over the past few years, he reported a gradual worsening of symptoms, including several accidental falls. Additionally, he exhibited tremors and motor clumsiness in fine hand movements. Consequently, the patient was admitted to our Dept.. During the neurological physical examination, we observed hypotrophy, weakness, and spontaneous fasciculations in the proximal muscles of both upper and lower limbs, as well as absent deep tendon reflexes in the lower limbs. In contrast, the patient exhibited brisk deep tendon reflexes in the upper limbs and a positive Hoffmann sign on the right. During hospitalization, we conducted tests for antiganglioside autoantibodies and antibodies to acetylcholine receptors (AChR), with mild positivity for the latter; no myasthenic clinical and/or electrophysiological were found. Magnetic resonance imaging of the brain and spinal cord showed no remarkable findings. A thigh magnetic resonance imaging revealed widespread atrophy of the quadriceps muscles bilaterally. Electromyography showed signs of chronic neurogenic distress with varying degrees of expression and findings of acute denervation and fasciculations in cervical and lumbar regions. Subsequently, genetic investigations for SMA, ALS (Amyotrophic Lateral Sclerosis), and SCA (Spinocerebellar Ataxia) were initiated. The analysis revealed homozygous deletion of the SMN1 gene and the presence of four copies of the SMN2 gene. Genetic investigations for ALS yielded negative results, while those for SCA are ongoing. Consequently, a diagnosis of SMA was made, and treatment with Nusinersen was initiated.

In the literature, there have been few descriptions of SMA patients with preserved deep tendon reflexes. These individuals were typically in the early stages of the disease and subsequently developed areflexia during follow-up. In our case, the patient exhibited (and continues to exhibit) an atypical clinical presentation of the disease after more than 10 years of illness.

This case underscores the importance of electrophysiological studies and genetic testing, especially in SMA patients with atypical clinical presentations.

Muscles in SMA

P12

FUS protein expression and distribution in the myopathology of 5q-associated spinal muscular atrophy type 3

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Spinal muscular atrophy (SMA) is a progressive, recessive neuromuscular disease characterized by significant reduction of SMN protein. This causes degeneration of lower motor neurons in the spinal cord and brainstem leading to weakness and muscle atrophy. Clinical severity is categorized in different subtypes (type 0-3), which is turn influenced by residual SMN level. While the genetic basis of SMA is well described, the tissue-specific molecular pathways underlying SMA are still not fully understood and identification of marker proteins (with pathophysiological relevance) in human vulnerable tissue such as skeletal muscle is still lacking.

To elucidate molecular markers in SMA-diseased muscle, we performed unbiased proteomics and transcriptomics on muscle biopsies derived from three SMA type 3 (walker) patients and moreover performed immunofluorescence studies on muscle biopsy specimen derived from five cases and on cultured myoblasts derived from three cases.

Combined proteomic and transcriptomic studies unraveled FUS (a DNA/RNA-binding protein and aggregation marker) as a potential molecular marker increased in SMA muscle. Results of our immunofluorescence studies showed a perimyonuclear disposition of FUS in a proportion of muscle cells compared to the immunoreactivity obtained in control samples.

Our data classify FUS as a protein involved in SMA-based myopathology and support the concept of skeletal muscle as a primary tissue target of SMA. Further studies are crucial to define the role of mis-localized FUS in SMA muscle.

P13

Longitudinal changes in compound muscle action potential (CMAP) and their association with motor function in children with infantile-onset SMA in ENDEAR/SHINE

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Compound muscle action potential (CMAP) is a validated, noninvasive, and simple to perform electrophysiological measurement providing physiologic information about motor units. To evaluate the potential of CMAP as a biomarker for therapeutic effects in spinal muscular atrophy (SMA), we examined longitudinal changes in CMAP in children with symptomatic SMA treated with nusinersen and their associations with motor function scores and the achievement of independent sitting.

Our analysis included participants with symptomatic infantile-onset SMA (symptom onset ≤6 months [mo], 2 *SMN*2 copies) who initiated nusinersen in the Phase 3, randomized, sham-controlled ENDEAR study (NCT02193074) or the open-label, long-term extension SHINE study (NCT02594124; 27 August 2019 data cut). Baseline (BL) characteristics at nusinersen initiation and longitudinal changes in peroneal and ulnar CMAP amplitudes after initiating nusinersen were analyzed. Associations between changes from BL in CMAP and Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) score at each visit were examined using Spearman correlation coefficients. Early CMAP response at Day 183 as a predictor of time to achieving independent sitting at later visits was examined using Fine-Gray subdistribution hazard models.

BL characteristics of 105 participants showed very low CMAP amplitudes and disease severity at nusinersen initiation. Mean peroneal and ulnar CMAP amplitudes were between 0.11 and 0.46 mV across three age groups with symptomatic SMA (<6 mo, ≥6 mo to <10 mo, and ≥10 mo to <23 mo). Both peroneal and ulnar CMAP amplitudes increased significantly after nusinersen initiation. The mean (SD) changes from BL for peroneal and ulnar amplitudes were 0.65 (0.75) and 0.26 (0.36) mV at Day 394, and 1.17 (1.22) and 0.49 (0.67) mV at Day 818, respectively. Increases were greater for participants who were younger vs. older at first nusinersen dose. Correlations between changes in CMAP and CHOP INTEND at Day 394 were 0.28 and 0.45 for peroneal and ulnar amplitude, respectively; the corresponding correlations were 0.46 and 0.42 at Day 818 (all $p<0.05$). Participants with increased CMAP at Day 183 were more likely to achieve independent sitting at later visits. Those with ≥0.5 mV increases in peroneal and ulnar CMAP at Day 183 had 1.9-fold (95% CI, 0.9–3.9) and 4.6-fold (95% CI, 1.3–16.7) increased probability of achieving independent sitting during the study, respectively.

Despite very low CMAP amplitudes at BL, children with symptomatic infantile-onset SMA treated with nusinersen showed continual increases in both peroneal and ulnar CMAP. Longitudinal changes in CMAP correspond with the observed improvement in motor function and are predictive of achieving independent sitting at later visits. CMAP may be a potential biomarker for therapeutic effects in infantile-onset SMA. Study Support: Biogen.

P14

SYNAPSE-SMA: A phase 2, randomised, double-blind, placebo-controlled, 2-way cross-over study of NMD670 in ambulatory adults with Spinal Muscular Atrophy Type 3 – Study design and status update

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Despite recent breakthroughs in the treatment of spinal muscular atrophy (SMA), preclinical and clinical studies indicate that in addition to motor neuron degeneration, the transmission of signals from nerve to muscle is dysfunctional in SMA and that this transmission deficit may persist despite treatment with currently available therapies. Therefore, drugs that facilitate neuromuscular transmission are candidate drugs for the treatment of SMA. NMD670 is a novel first-in-class neuromuscular transmission enhancer working through selective inhibition of the skeletal muscle chloride channel 1 (ClC-1) that is being developed by NMD Pharma for the treatment of neuromuscular disorders characterized by neuromuscular transmission dysfunction.

The SYNAPSE-SMA study is a proof-of-concept, phase 2, randomised, double-blind, placebo-controlled, 2-way crossover study to evaluate the efficacy, safety, and tolerability of NMD670 in ambulatory adults with type 3 SMA. An estimated 54 ambulatory adults with type 3 SMA will be randomized at approximately 25 sites in North America and Europe. Eligible participants will be randomized to receive NMD670 or placebo twice daily for 21 days and will cross over to the opposite treatment after a 7-day washout period. The primary endpoint is the change from baseline versus placebo in total distance walked during the 6-minute walk test. Secondary endpoints include safety and tolerability, and the change from baseline versus placebo in muscle strength (handheld dynamometry), motor function (Revised Hammersmith Scale), fatigue (endurance shuttle nine-hole peg test and fatigue index calculated from 6-minute walk test) and neuromuscular transmission (single fiber electromyography). Exploratory outcomes include pharmacokinetics and change from baseline versus placebo in patient reported fatigue (Fatigue Severity Scale) and quality of life (Individual Neuromuscular Quality of Life Scale).

The first subject was enrolled in September 2023 and NMD Pharma will provide a status update on enrolment during the 4th Scientific International Congress on SMA in Ghent, March 2024.
NMD Pharma plans to evaluate NMD670 in other types of SMA, including pediatric and non-ambulatory populations depending on the results obtained from the current proof of concept study.

P15

MANATEE: GYM329 (RO7204239) in combination with risdiplam treatment in patients with SMA

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Risdiplam (EVRYSDI®) is a centrally and peripherally distributed, oral survival of motor neuron 2 (*SMN2*) pre-mRNA splicing modifier approved for the treatment of spinal muscular atrophy (SMA) in 100 countries worldwide. GYM329 is an investigational, recycling and antigen-sweeping monoclonal anti-myostatin antibody (myostatin is a negative regulator of skeletal muscle growth). In preclinical studies, the combination of GYM329 with an *SMN2* splicing modifier demonstrated improved muscle size and strength compared with *SMN2* splicing modifier treatment alone.
MANATEE (NCT05115110) is a multicentre, two-part, randomised, placebo-controlled, double-blind study investigating the effect of GYM329 in combination with risdiplam in treatment-naïve and non-treatment-naïve patients.
Here we present information about the MANATEE study design.

Recently, the eligibility criteria of MANATEE have been broadened and the target enrolment increased to include ~259 participants. Part 1 (target enrolment n~93) will assess safety, tolerability and pharmacokinetics/pharmacodynamics of different GYM329 doses in combination with risdiplam. Part 1 now includes a non-ambulant cohort. Part 2 (target enrolment n~166) will assess efficacy and safety of the Part 1-selected dose of GYM329 in combination with risdiplam. Patients aged 2–25 years are now eligible to enrol in Part 2. The primary endpoint of Part 2 will be the change from baseline in the Revised Hammersmith Scale (RHS) total score at Week 72. Secondary efficacy endpoints include changes from baseline in the 32-item Motor Function Measure; time taken to rise from the floor (RHS Item 25); time taken to walk/run 10 meters (RHS Item 19); and lean muscle mass as assessed by dual-energy x-ray absorptiometry (patients aged ≥5 years).

Risdiplam has demonstrated clinically meaningful benefits in patients with SMA. GYM329 in combination with risdiplam may have a complementary effect on the improvement of patients' motor function.

P16

SMN deficit restricted to skeletal Muscle Stem Cells (MuSC) triggers selective loss of alpha motor neurons

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Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder mainly affecting children, characterized by progressive degeneration of motor neurons (MN) and muscle atrophy. In most cases, SMA is due to homozygous mutations in the *Survival of Motor Neuron* gene (*SMN1*), leading to SMN protein deficit. Recently approved therapeutic approaches including AAV-mediated *SMN1* gene replacement and modulation of *SMN2* transcript splicing by antisense oligonucleotides or small molecules have proven unprecedented therapeutic benefits raising great hopes for the treatment of this devastating neuromuscular disease. However, uncertainties remain regarding the long-term efficacy of these treatments and the involvement of peripheral tissues in the pathology.

We performed an in-depth analysis of the role of SMN in the regulation of muscle stem cells (MuSC) during early postnatal growth and in the adult muscle. We observed that the number of quiescent PAX7+ MuSC in muscles from juvenile SMA type 2 patients was significantly reduced compared to controls. By characterizing postnatal myogenesis of SMA mice and overexpressing SMN in wildtype mice, we showed that SMN level regulates MuSC progression in the myogenic lineage and establishment of quiescent MuSC pool. Using an inducible conditional knockout mouse model, *Pax7$^{CreERT2/+}$;Smn floxed* mice, we observed that ablation of one or both *Smn* alleles induced quiescent MuSC apoptosis, demonstrating that high levels of SMN are necessary for quiescent MuSC pool maintenance. We showed that depletion of SMN-deficient MuSC induces a rapid remodeling of the neuromuscular junctions (NMJ), and at longer a selective loss of alpha MN while gamma-MN were not affected. Overall, our study demonstrates that SMN insufficiency in quiescent MuSC could induce a non-cell autonomous loss of MN. MuSC are therefire crucial therapeutic targets to ensure the preservation of the neuromuscular system of treated patients over the long term. These data provide new insights into the pathophysiology of the disease that have major implications for the design of next generation therapies.

Treatments

P17

Exon7 Targeted CRISPR-Prime Editing Approaches for SMN2 Gene Editing in Spinal Muscular Atrophy

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Spinal Muscular Atrophy (SMA) is a neurodegenerative disease characterized by loss of alpha motor neurons. There are 2 genes, *SMN1* (Survival Motor Neuron 1) and *SMN2*, which are associated with SMA disease and these genes express SMN protein. The SMN2 gene expresses less than 10% of the functional SMN protein. Although the *SMN1* and *SMN2* genes are considered nearly identical, they differ from each other by only 11 nucleotides. The most important change between *SMN1* and *SMN2* is the inhibition of exon 7 participation as a result of C-T transformation at 840 nucleotides in the *SMN2* gene. This nucleotide change causes the exonic splicing enhancer, which operates on exon 7, to be converted into a silencer. For this reason, the elements involved in the splicing phase cannot bind to the binding site and the production of SMN protein that has lost its function, which does not contain exon 7 at a rate of 80-95%, takes place. CRISPR technology, which adds a new dimension to genetic engineering and gene therapies, allows the treatment of many genetic diseases. In terms of SMA, some previous studies in the literature prove that it is possible to treat SMA with the CRISPR strategy. CRISPR-Prime Editing (PE) technology is a next-generation gene editing approach that precisely enables a variety of genomic modifications without the need for double-strand breaks or donor DNA sequences. CRISPR Prime Editing-mediated gene editing for SMA was reported for the first time in our study (Odabaş et al., BioRxiv, 2022). The c.840 T-C transition and c.859 G-C transformations in the *SMN2* gene and the simultaneous correction of these point mutations with a single pegRNA will be examined for the first time in this project. We show that CRISPR-PE systems can increase *SMN2* gene activity and SMN protein expression by regulating c.840 T-C transition and c.859 G-C transformations, enabling exon 7 participation. We demonstrated in our preliminary work as a proof-of-concept the efficiency and stability of the Prime Editing method of modifications in *SMN2* genes investigated in SMN-low Jurkat cells, primary SMA type I, and type II fibroblast cells.

P18

Interim clinical and safety results from the ongoing open-label phase 4 RESPOND study evaluating nusinersen in children with spinal muscular atrophy previously treated with onasemnogene abeparvovec

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RESPOND (NCT04488133) is a single-arm study evaluating nusinersen (NUS) treatment in children with SMA who previously received onasemnogene abeparvovec (OA) and have suboptimal clinical status at enrollment per the investigator. We report baseline (BL) characteristics and interim clinical outcomes and safety findings.
RESPOND participants (pts) had ≥1 *SMN2* copy, were aged ≤36 mo, received OA ≥2 mo before their 1st dose of 12-mg NUS, and had suboptimal clinical status in ≥1 of 4 domains at BL: motor function, swallowing/feeding ability, respiratory function, other. Changes from BL in suboptimal clinical status and in total HINE-2 and CHOP INTEND scores were examined in pts who reached Day 183 assessment by the time of the data cut (15Nov2022). Analyses of clinical outcomes were stratified by age at 1st NUS dose (≤9 vs >9 mo) and *SMN2* copy number. Safety data were evaluated as aggregate in all pts who received ≥1 NUS dose.

At the time of the data cut, the 38 enrolled and dosed pts were on study for a median of ~230 days. Of 29 pts who reached Day 183, 14 were ≤9 mo at 1st NUS dose and had 2 *SMN2* copies (group 1; median [range] age: 7.7 [3.4–9.8] mo), 12 were >9 mo with 2 *SMN2* copies (group 2; 16.3 [11.0-33.3]), and 3 were >9 mo with 3 *SMN2* copies (group 3; 30.8 [29.2–35.7]). All were symptomatic at the time of OA dosing (median [range] age at OA: 1.7 [0.7–5.1] mo in group 1; 2.7 [0.8–6.9] in group 2; 17.5 [13.6–24.3] in group 3). At BL, most had low ulnar CMAP amplitude (≤1 mV), and 93% of pts in group 1, 92% in group 2, and 33% in group 3 had investigator-reported suboptimal clinical status in ≥2 domains. At Day 183, most pts with suboptimal motor function at BL reported improvement (92% in group 1, 91% in group 2, 100% in group 3). Investigators most often reported no change in suboptimal swallowing/feeding ability or respiratory function, but some reported improvement. Mean (SD) change from BL to Day 183 in total HINE-2 scores was 5.4 (2.6) in group 1 and 5.2 (2.7) in group 2 (not calculated in group 3 due to small sample size). Mean (SD) scores at Day 183 reached 8.6 (3.5) and 13.5 (5.8) in each group, respectively. On average, pts who were unable to sit without support improved on CHOP INTEND. Mean (SD) changes in CHOP INTEND were 6.7 (6.8) and 3.6 (3.9) in nonsitters in group 1 and group 2. Adverse events (AEs) occurred in 31/38 (82%) pts, most commonly infections/infestations (63%) and respiratory, thoracic, and mediastinal disorders (26%). Two (5%) pts had an AE determined by the investigator to be related to NUS (mild proteinuria), which resolved. Thirteen (34%) had serious AEs; none related to NUS. Additional data will be presented.

Most pts showed suboptimal clinical status in multiple domains at BL. At Day 183, the majority of pts receiving NUS showed improvements in motor function. Reported interim safety outcomes are consistent with the established safety profile of NUS. Study Support: Biogen.

P19

Risk factors for adrenal suppression and steroid dependence post onasemnogene abeparvovec infusion in Spinal muscular atrophy type 1

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Onasemnogene abeparvovec (OA) is a licenced treatment for Spinal Muscular Atrophy Type 1 in the UK. [1] Steroid treatment is required to reduce immune-mediated hepatotoxicity. [2] Prednisolone is commenced at 1mg/kg/d, maintained for 30 days, then gradually weaned. Dose is doubled to 2mg/kg/d if complications occur including transaminitis, thrombocytopenia and myocarditis. Steroid weaning safety protocols recommend cessation of steroids once the adrenal axis recovers, detected by cortisol investigations. In our cohort, we are observing prolonged steroid requirement with multiple failed adrenal suppression tests.
To assess if any patient or treatment factors contribute to prolonged steroid requirement. In identifying these, potentially improve management of hepatotoxicity risk and adverse events due to long-term steroid use. [2]

We carried out an audit of all our 25 patients who have received OA under our care, 17 who have completed and 8 with on-going steroid treatment.

Completed steroid treatment and <13.5kg; n=15, Age; Median 5months (m), range 1-20m. Weight; median 7kg, range 4.1-10.9kg. (Steroid requirement; median 6m, range 3-12m). Completed steroid treatment and >13.5kg, n=2, age 51m, 86m; weight 15.5kg,19.5kg respectively. (Steroid requirement; 7m and 11m). For all 17 patients there was no correlation between age (p=0.576) or weight (p=0.542) at time of infusion. Twelve patients had 2 *SMN2* copy numbers, (steroid requirement; median 8m, range 3-12m). 5 had 3 *SMN2* copy numbers, (steroid requirement; median 5m, range 4-7m) not statistically significant (p=0.1248).
Five patients required higher dose prednisone due to transaminitis with two patients also having subclinical thrombocytopenia, (steroid requirement; median 8m, range 4-12 m). For patients who remained on lower dose steroids, (steroid requirement; median 6m, range 3-12m).
Patients previously treated with nusinersen, n=8. Age; median 7m, range 3-86m. Weight; median 7.6kg, range 5.3 - 19.5kg, (steroid requirement; median 8.5m, range 4-12m).
Treatment naïve, n=9. Age; median 5m, range 1-16m. Weight; median 8.5kg, range 4.1-10.9kg, (steroid requirement; median 5.5m, range 3-12m).
8/25 patients were still steroid dependent, 4 were on steroids for ≥12m (12,14, 20, and 22m), with 3 out of 4 <13.5 Kg at infusion. The fourth patient was >13.5 Kg at infusion, the eldest patient and required additional immunosuppressive treatment despite optimal steroid treatment due to severe hepatotoxicity.

Our preliminary data shows potential risk factors for prolonged steroid treatment align with increased disease severity, further research in larger cohorts is vital to increase understanding further. No single factor was proven to prolong steroid use thus highlighting the importance of vigilant monitoring post OA infusion and reduction of steroids as soon as clinically safe.

P20

Novel copy-specific differential methylation patterns across *SMN2*: comprehensive analysis of DNA methylation as potential disease modifier

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Our understanding of the genetic variation that causes heterogeneity in spinal muscular atrophy (SMA) disease severity and treatment response remains limited. SMA can progress at strikingly different rates even in patients with the same copy number of the disease modifying gene *survival motor neuron 2* (*SMN2)*. This suggests not all *SMN2* copies are equally functional or active. One possible cause for this variability is DNA methylation, which is known to directly influence gene activity. Previous research showed that DNA methylation in the promoter of *SMN2* can differ between patients with equal *SMN2* copy number. However, comprehensive data from large cohorts and across the full *SMN2* gene is still missing. Therefore, we performed targeted, long-read Nanopore sequencing of the *SMN* locus in blood or fibroblasts of 32 SMA patients with varying *SMN2* copy numbers. The amplification-free nature of long-read sequencing allows for direct DNA methylation analysis without prior conversion of the DNA as used by traditional methods. In addition, the long reads, spanning tens to hundreds of kilobases, enable *SMN2* copy-specific methylation analysis. In all patients, we detected hypermethylation across nearly the entire *SMN2* gene and hypomethylation in the *SMN2* promoter region. In addition, we identified multiple regions of interest (ROIs) across the gene with variable levels of decreased methylation, including the 3' untranslated region (UTR) in blood and the region containing the *SMN*-regulating long non-coding RNA *SMN-AS1* in fibroblasts. *SMN2* copy-specific methylation analysis furthermore revealed that methylation differs per *SMN2* copy at multiple other CpG sites, suggesting that *SMN2* copies can be functionally different, even within the same individual. These sites were added to our selected ROIs. Next, we will perform amplicon-based bisulfite sequencing for these ROIs in 400 patients. This will allow us to confirm our initial results and to determine if differential DNA methylation in *SMN2* is associated with disease severity, treatment response or *SMN2* mRNA expression.

P21

Long-term muscle-specific overexpression of DOK7 in mice using AAV9-tMCK-DOK7

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Neuromuscular junction (NMJ) dysfunction underlies several diseases, including the motor neuron diseases (MNDs) spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS). Molecular pathways governing NMJ stability are therefore of interest from both biological and therapeutic perspectives. Muscle-specific kinase (MuSK) is necessary for the formation and maintenance of post-synaptic elements of the NMJ, and downstream of tyrosine kinases 7 (DOK7) is crucial for activation of the MuSK pathway. Overexpression of DOK7 using AAV9 has been shown to ameliorate neuromuscular pathology in pre-clinical disease models of SMA and ALS. However, long-term consequences of DOK7 expression have been sparsely investigated and targeted overexpression of DOK7 in skeletal muscle yet to be established.

We developed and characterized a novel AAV9-DOK7 facilitating forced expression of DOK7 under a skeletal muscle-specific promoter. AAV9-tMCK-DOK7 was systemically delivered to newborn wildtype mice that were monitored over 6 months.

DOK7 overexpression was restricted to skeletal muscles. Body weight, blood biochemistry, and histopathological assessments were unaffected by AAV9-tMCK-DOK7 treatment. In contrast, forced expression of DOK7 resulted in enlargement of both the pre- and post-synaptic components of the NMJ, without causing denervation.

We conclude that muscle-specific DOK7 overexpression can be achieved in a safe manner, with the capacity to target NMJs *in vivo*

P22

Clinical evolution of children with SMA 5q treated with specific drugs

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5q SMA is the most common form of SMA and is associated with the mutation of the SMN1 gene. In recent years, the disease has had specific approved pharmacological therapies that have modified the natural course of its evolution with the consequent appearance of improved phenotypes and offering a better quality of life to children with this pathology.

Observational, descriptive, retrospective and cross-sectional study. Patients with a diagnosis of 5q SMA treated with specific drugs (Spinraza®, Zolgensma®, Evrysdi®) for at least 6 months were included, from November 2019 to September 2023. Variables: Data demographics, Types of SMA, Genetic study, Complications, Clinical evolution (motor, respiratory, swallowing, nutritional, osteoarticular, cardiological), Specific drugs (Spinraza®, Zolgensma®, Evrysdi®). descriptive statistics were used 57 patients with a diagnosis of SMA 5q were treated, of which 18 patients were excluded because they did not meet the inclusion criteria. 39 patients entered the study, of which 56.4% (22/39) were male and 43.6% were female. According to the type of SMA: 15/39 (38%) are Type I, 21/39 (54%) Type II and 3/39 (8%) Type III. All patients (39/39) had 0 copies of the SMN1 gene. Regarding the number of copies of the SMN 2 gene: All SMA Type I with 2 copies (15/15), SMA Type II with 2 (2/21) and 3 copies (19/21), and all Type III with 3 copies (3/3). The median age at genetic diagnosis was 23 months. The median time to start specific therapy post-genetic diagnosis was 12 months. The types of drugs administered were: Spinraza® in 2 patients with SMA I, Zolgensma® in 2 patients with SMA Type I, and Evrysdi® in 13 patients with SMA Type I. I, 20 patients with SMA Type II and 3 with SMA Type III. 2 patients with SMA Type I switched from Spinraza® to Evrysdi®. Motor evolution: All patients (100%), regardless of the type of SMA and the clinical condition, at the beginning of the drug, presented improvement in motor and respiratory functions to a variable degree. Nutritional evolution: The majority of SMA Type I (13/15) started therapy with some degree of malnutrition, of which 9/13 improved their nutritional status and 4/13 with severe malnutrition and the remaining 2/15 remained with good nutritional status. Of the patients with Type II SMA at the beginning of treatment: 4/21 were overweight, 13/21 with variable degree malnutrition and 4/21 with normal nutritional status, over time there was improvement in the malnourished and the rest remained the same. All patients with Type III SMA (3/3) were obese at the beginning, then 2 became overweight and 1 became normal. Swallowing evolution: all SMA Type I patients presented swallowing alteration: 5/15 of moderate degree and 10/15 of severe degree, improving with therapy, only those of moderate degree and the rest remained the same. The majority of SMA Type II (19/21) presented with normal swallowing and remained the same, the rest without changes. All SMA Type III (3/3) maintained normal swallowing. Osteoarticular evolution: At the beginning of therapy: SMA Type I mostly without scoliosis (13/15), SMA Type II the majority (18/21) with scoliosis of variable degree, and SMA Type III all with moderate to severe scoliosis . Over time all scoliosis worsened. 1 underwent surgery and 4/39 have indications for surgery. The majority of patients (35/39) with some degree of hamstring retraction and bilateral Achillea, only 4 without retractions. Cardiological evolution: All patients evaluated and treated (100%) with normal cardiac function, with no changes observed. no patient died. all patients (39/39) received motor physiotherapy, respiratory physiotherapy and nutritional support.

Specific drugs for SMA 5q positively modify the natural clinical evolution of the disease, either stabilizing or improving it regardless of the patient’s previous clinical condition, and thus providing a better quality of life to these children.

P23

3D-stem cell spinal cord model to study timing and cell type specific molecular consequences of risdiplam-like treatment

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The currently approved therapeutic approaches for SMA leverage the possibility of restoring SMN protein level either through mRNA splicing modification or gene replacement. Among them, risdiplam, daily orally administered already in newborns, is a small molecule that interferes with the recruitment of splicing transcription factors at exon7 of *SMN2* to promote its retention. Despite the impressive therapeutic effects, important open questions exist on the use of this drug such as a clear profile of molecular consequences on targets other than *SMN2*. 3D cellular models of the primary target organ derived from disease and healthy subjects, the latter in principle non-responders to on target treatment effects, can help to address those questions *in vitro*. Further, better understanding of long-term treatment consequences and therapeutic window optimization if starting treatment at different time points of organoid maturation could be identified

To test the molecular and phenotypic consequences using a risdiplam derived compound in a human *in vitro* model of spinal cord development.

We generated and phenotypically characterized by immunofluorescence human SMA type 1 spinal cord organoids from induced pluripotent stem cells (iPSCs) of n=3 subjects and from 3 healthy controls. We used omics approaches, including single cells, bulk transcriptomics, and multi-electrodes array analysis. Treatment was started at different time points within the first 80 days of organoid development, which parallels the first trimester post conception.

Prelliminary data of long-term Risdiplam-like compound *in vitro* treatment is well-tolerated, supporting the idea that SMA organoids represent a reliable model to explore drug kinetics and efficacy. Further, Risdiplam-like compound if administered when neuronal maturation starts, models ~ 15% of disease affected genes. Moreover, single cell analysis underlined a robust alteration of cell type frequency depending on organoid treatment start.

Overall, our study contributes to the optimization of Risdiplam therapy and to the precise identification of its molecular signature in different developmental phases.

P24

Gene therapy of spinal muscular atrophy with progressive myoclonic epilepsy (SMA-PME) by intracerebroventricular administration of an AAV9-ASAH1 vector in mice

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Spinal muscular atrophy with progressive myoclonic epilepsy (SMA-PME) is an autosomal recessive disorder caused by mutations in the *ASAH1* gene, which codes for acid ceramidase (ACDase), a lysosomal enzyme that catalyses the bioactive lipid ceramide into sphingosine and fatty acid. The disease is progressive, starting during childhood with muscle weakness and/or myoclonic seizures, and leads to death in most cases at teenage. To date, there is no curative treatment and therefore a clear unmet medical need. In the present study, we evaluated a gene therapy approach in a severe mouse model of acid ceramidase deficiency by delivery of an AAV9 vector expressing human ACDase into the cerebrospinal fluid (CSF). We report that intracerebroventricular administration of the vector in newborn *Asah1*$^{P361R/P361R}$ mice prolonged the lifespan of animals and ameliorated body growth and motor activity. The treatment rescued the signs of disease in the central nervous system, but not completely in peripheral organs. Phenotype correction correlated with vector biodistribution and transgene expression in tissues. Our findings suggest that CSF delivery of an AAV9-ASAH1 vector may represent a therapeutic strategy for SMA-PME patients.

P25

Risdiplam treatment in symptomatic early-onset SMA patients led to clinically meaningful improvement, single-center experience

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Spinal muscular atrophy (SMA) is a rare genetic neuromuscular disorder characterized by the degeneration of motor neurons, leading to muscle weakness and atrophy. The development and approval of disease-modifying treatments have dramatically changed disease progression in patients with SMA. Risdiplam became available in North Macedonia in 2021 for the treatment of genetically confirmed SMA patients irrespective of age and disease severity. The aim was to assess the improvements in motor function in symptomatic infantile-onset SMA patients under risdiplam treatment in North Macedonia.

This observational retrospective study utilized patient hospital files from the only reference center for SMA management in North Macedonia, the University Clinic of Pediatric Diseases, Skopje. Data collected from the patient files included demographic and clinical features. The total CHOP-INTEND score was recorded before the initiation of treatment and one year after treatment started. Drug-related adverse events were also monitored and recorded throughout the period. Data were collected during routine patient visits as real-world outcome data.

The results showed that 3 out of 10 early-onset SMA patients were receiving risdiplam for a duration of 16 months to 2.5 years. The average age of risdiplam-treated patients was 3.17 years, with an age range of 2.7 to 4 years. The average age at symptom onset and genetic diagnosis was 11 months. The time between genetic confirmation and starting treatment with risdiplam was an average of 2 months [range from 1 to 4 months]. Two out of three patients with SMA Type 1 and Type 2 were female. Two patients were able to sit, while one patient was non-sitter. The patients had 2 to 3 copies of the SMN2 gene. Scoliosis was present in all three patients. During this period, all patients maintained the ability to swallow and took risdiplam orally. After 12 months of treatment, patients' motor function improved, as indicated by the total CHOP-INTEND and HFMSE scores. The patient #1 (SMA Type 2, 3 SMN2 copies) and patient #2 (SMA Type 1, 2 SMN2 copies) showed an increase in the total CHOP-INTEND score from 18 to 36 (100% improvement), and from 14 to 18 (28.5% improvement), respectively. The patient #3 (SMA Type 2, 3 SMN2 copies) showed an increase in the total HFMSE score from 22 to 24 (9% improvement). No adverse events were observed during the period.

These findings suggest that risdiplam treatment in symptomatic pediatric SMA patients can lead to significant and clinically meaningful improvements in motor function. In addition, they suggest that risdiplam is safe to use, as no adverse effects have been observed. Further research and longer follow-up periods are needed to fully assess the efficacy and safety of risdiplam in this population.

P26

Nusinersen treatment for spinal muscular atrophy: Effects on excitatory synaptic integrity and microgliosis

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Spinal muscular atrophy (SMA) is caused by insufficient levels of survival motor neuron (SMN) protein. The restoration of SMN protein levels by the antisense oligonucleotide (ASO) nusinersen has transformed the clinical outcome of SMA patients. However, its ability to counteract the disease is still limited. In order to find which targets are not efficiently reached by nusinersen, it is essential to elucidate the cellular and molecular changes underlying its pharmacological action. The aim of this study is to analyze the impact of nusinersen treatment on distinct hallmarks of SMA cellular pathology in the SMNΔ7 mouse model. We examine the effect of this therapy on motor neuron (MN) deafferentation, particularly on cholinergic C-boutons, which play a critical role in neuronal excitability, and on glial activation.

SMNΔ7 mice were treated at the day of birth with a single intracerebroventricular (ICV) injection of either nusinersen (6.5 mg/kg) or saline solution. The weight, motor function and survival of mice were assessed to determine the clinical effect of the treatment. To analyze the status of synaptic inputs onto MNs and microgliosis, lumbar spinal cords of post-natal day (P) 14 mice were processed for immunohistochemistry and studied by confocal microscopy.

Nusinersen treatment significantly increased body weight, improved motor performance and extended survival (average days 40.21±11.87) of SMNΔ7 mutant mice. SMA spinal cord MNs undergo a loss of synaptic inputs. The immunohistochemical analysis revealed that, the cholinergic deafferentation observed in mutant mice is partially prevented by nusinersen. This was evidenced by an increased density of a number of proteins associated to C-boutons (vesicular acetylcholine [ACh] transporter, muscarinic M2 ACh receptors, Y172, Sigma-1 receptor and Neuregulin-1). The glutamatergic synaptopathy observed in SMNΔ7 mutant mice MNs was also ameliorated by this treatment. Moreover, MN deafferentation in mutant mice was accompanied by morphological changes in microglial cells, which became bigger and more ramified, suggesting the acquisition of a reactive phenotype. These microglial changes did not appear to be ameliorated by nusinersen treatment.

ICV administration of nusinersen at birth ameliorated, but did not utterly prevent, the excitatory synaptopathy inherent to MNs of SMNΔ7 mutant mice. Additionally, nusinersen was unable to prevent the microglial reaction associated with SMA pathology. These cellular outcomes go in line with the highly remarkable, although partial, motor behavior amelioration of these mice after the ASO treatment. Therefore, pre-natal approaches or therapies combining nusinersen with SMN-independent drugs should be investigated to achieve a better MN preservation.

P27

FIREFISH Parts 1 and 2: FIREFISH Parts 1 and 2: 48-month safety and efficacy of risdiplam in Type 1 SMA

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Risdiplam (EVRYSDI®) is a centrally and peripherally distributed, oral survival of motor neuron 2 (*SMN2*) pre-mRNA splicing modifier that increases and sustains the levels of functional SMN protein. Risdiplam has been approved for the treatment of spinal muscular atrophy (SMA) in 100 countries worldwide.

FIREFISH (NCT02913482) is an open-label, two-part, multicentre clinical study of risdiplam in children with Type 1 SMA and two *SMN2* gene copies (inclusion criteria: 1–7 months of age at enrolment). FIREFISH Part 1 assessed the safety, tolerability and pharmacokinetics/pharmacodynamics (PK/PD) of different risdiplam doses. Pivotal Part 2 assessed the safety and efficacy of risdiplam over 24 months at the dose selected from Part 1. Thereafter, children entered a 3-year open-label extension phase and continue to receive risdiplam at the pivotal dose.

Pooled safety and efficacy data were available from 58 enrolled infants who received risdiplam treatment (Part 1 high-dose cohort, n=17; and Part 2, N=41). As of the cut-off date (23 Nov 2021), there were no treatment-related adverse events leading to withdrawal, no additional deaths and no additional children meeting the definition of permanent ventilation since Month 24. At Month 36, 84% of children were alive and did not require permanent ventilation. Children either maintained or improved their motor skills in terms of developmental milestones and motor function between Months 24 and 36, which is not observed in natural history. Here we present longer-term pooled safety and efficacy data from children who have received risdiplam at the pivotal dose for at least 48 months.

FIREFISH Parts 1 and 2 are ongoing globally and will provide further safety and efficacy data of risdiplam in Type 1 SMA.

P28

Exploration of the use of risdiplam administration in patients with SMA who previously received gene therapy

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Risdiplam (EVRYSDI®) is a centrally and peripherally distributed, oral survival of motor neuron 2 (*SMN2*) pre-mRNA splicing modifier that increases functional SMN protein levels. Risdiplam has been approved for the treatment of spinal muscular atrophy (SMA) in 100 countries worldwide. Onasemnogene abeparvovec (OA; ZOLGENSMA®) is an intravenously administered, adeno-associated virus vector-based gene therapy for the treatment of SMA. OA delivers a functional copy of the human *SMN1* gene to target motor neurons. The JEWELFISH trial (NCT03032172) is an ongoing, multicentre, open-label study evaluating risdiplam in a broad patient population with Types 1–3 SMA, who have previously received other disease-modifying therapies. An exploratory analysis of JEWELFISH data showed that those previously treated with OA had increased levels of SMN protein (n=12) and no new safety signals (n=14) over 24 months of risdiplam treatment. Patients <2 years of age (n=3) who were previously treated with OA and who received risdiplam for 24 months, maintained or gained the ability to sit without support.

Here we describe the study design of two new single-arm trials that will evaluate the safety and efficacy of risdiplam administration in patients with SMA, <2 years of age at enrolment, with two *SMN2* copies, who were previously treated pre- or post-symptomatically with OA.

The first study will evaluate the safety and efficacy of prophylactic risdiplam treatment after the administration of OA. The second study will evaluate the safety and efficacy of risdiplam administered in patients who have experienced a plateau or decline in motor function after OA gene therapy. Assessments in the studies will include the Gross Motor Scale of the Bayley Scales of Infant and Toddler Development, third edition, the Oral and Swallowing Abilities Tool, the Peabody Developmental Motor Scales, and safety assessments.

These studies will be enrolling soon at multiple sites globally. Results are expected to provide important information on the safety and efficacy of risdiplam treatment after OA gene therapy.

P29

Long-term comparative efficacy and safety of risdiplam versus nusinersen in children with Type 1 SMA

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Risdiplam and nusinersen are disease-modifying therapies (DMTs) approved for the treatment of spinal muscular atrophy (SMA). There are no long-term data on relative efficacy and safety of these DMTs. In the absence of head-to-head trials, indirect treatment comparisons adjusted for cross-trial differences can inform treatment decision-making.
The objective of this study is to compare long-term efficacy and safety of risdiplam versus nusinersen in children with Type 1 SMA.

Patient-level risdiplam data from 58 children in FIREFISH (Parts 1 and 2; NCT02913482) were compared with published nusinersen data from 81 children in SHINE (ENDEAR cohort; NCT02193074).
Matching-adjusted indirect comparisons were used to compare outcomes between risdiplam and nusinersen groups, adjusting for age at first dose, disease duration and Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) total score at baseline. Cox proportional-hazards models were used to compare overall survival, event-free survival and the times to Hammersmith Infant Neurological Examination, Module 2 (HINE-2) motor milestone responses, CHOP-INTEND responses, and the occurrence of any serious adverse event (SAE).

After matching, relevant baseline characteristics were identical across groups. The effective sample size for risdiplam was 40.6. Median follow-up was 3 years (range 2.5–4.5). Compared with the nusinersen group, the risdiplam group had 78% lower rate of death (95% confidence interval [CI] 53–96%), 81% lower rate of death or permanent ventilation (95% CI 65–93%), 57% lower rate of SAEs (95% CI 42–68%), and higher rates of HINE-2 and CHOP-INTEND responses. While adjustments were made for known prognostic factors, as in any non-randomised comparison, results may be confounded by unobserved baseline differences between groups.

Risdiplam was associated with longer survival, higher rates of motor function responses and lower rates of SAEs than nusinersen in children with Type 1 SMA. This comparative analysis leverages the longest follow-up currently available from two robust clinical trial sources. Additional data sources should be consulted to expand on these findings.

P30

A Phase 1 Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of BIIB115: An Anti-sense Oligonucleotide for Spinal Muscular Atrophy with Long Interval Dosing

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BIIB115 is an investigational, intrathecally administered antisense oligonucleotide (ASO) that has been shown in nonclinical studies to modify splicing of the SMN2 gene, leading to an increase in full length SMN protein similarly to nusinersen. The ASO has a novel modification in the chemical backbone, which may allow for long interval dosing.

Biogen is currently evaluating the safety, tolerability, and pharmacokinetics of BIIB115 in a Phase 1 study. In Part A of this study, 38 healthy adult men received a single injection of placebo or BIIB115 at 4 ascending dose levels. Part B is being initiated at EU sites to evaluate BIIB115 in children with SMA in an open-label study design. This presentation provides a study design overview for Part B.

In Part B, participants will be administered 2 doses of BIIB115 over the study duration and followed for 2 years. Up to 2 dose levels will be assessed. Dosing will be staggered by age such that older participants (aged 2-12 years) will be enrolled prior to younger participants (aged 0.5 to 2 years) for each dose level. Eligible participants are aged 0.5 to 12 years, have a genetic diagnosis of SMA with ≥1 *SMN2* copy, have received treatment with onasemnogene abeparvovec ≥180 days prior to enrollment, and have potential for improvement due to suboptimal clinical status. The primary objective is to evaluate safety and tolerability, with a primary endpoint of adverse events incidence over the study duration. Secondary endpoints will evaluate pharmacokinetic parameters and exploratory endpoints will evaluate biomarkers (including neurofilaments) and clinical outcome measures (including WHO motor milestones, HINE-2 motor milestones, CHOP INTEND, HFMSE, and RULM, as appropriate for age). The study schedule will include a 24-hour inpatient stay for monitoring after each dose, and regular follow-up visits for clinical assessments including blood draws. The results of this Phase 1 study will inform the optimal dosing regimen for BIIB115 and the design of future trials in SMA.

P31

Long-term follow-up of non-sitter patients aged 16 years and older with 5q spinal muscular atrophy treated with risdiplam

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Risdiplam has been approved for the treatment of patients with 5q spinal muscular atrophy (SMA) in 2019 in Europe, but long-term follow-up real-world data in type 2 non-sitter patients are lacking. We aim to describe our experience of treatment with risdiplam after 36 months of follow-up in a series of type 2 non-sitter patients.

Type 2 SMA patients older than 16 years were offered risdiplam through the expanded access program (EAP) (NCT04256265). Patients were followed up for safety and efficacy with a battery of scales and clinical measures at 12, 24 and 36 months.

Six non-sitter patients (17 – 46 years old) were treated with risdiplam. One patient reported recurrent adverse events (AE) (dyspepsia and headache) and discontinued the medication after 14 months of treatment. No other clinically significant AEs were reported. After one year of treatment, all patients showed clinically meaningful improvements in at least one scale and none of them showed any clinically meaningful deterioration. Two patients showed a clinically meaningful increase in the body mass index (BMI) (>5%) and two others in the revised upper limb module (RULM) (>2 points). Moreover, five patients experienced clinically meaningful improvements in the egen klassifikation 2 scale (EK2) (>2 points), including the motor (axial and upper limbs), bulbar (speech and swallowing) and respiratory (coughing) domains. Four subjects achieved at least one of the goals set with the goal attainment scale (GAS). After two years of treatment, 4 of the 5 patients maintained the clinical improvements achieved during the first year of treatment. The fifth patient continued to improve in the 24-months follow-up visit, increasing 3 points in the revised upper limb module (RULM), 4 points in EK2 (3 of them in the bulbar domain) and 27% in the body mass index (BMI). The results of the 36-months visit will be also presented.

This study suggests that long-term treatment with risdiplam is safe and well-tolerated. Moreover, most patients experienced clinically significant improvements after one year of treatment with one patient showing a sustained remarkable improvement in the bulbar function after 2 years of treatment.

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Spinal muscular atrophy (SMA) patient survey of Risdiplam: Impact on the SMA patients

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Risdiplam SMA treatment has been available since 2020 all patient with SMA via a early access to medicines scheme. In March 2022 Risdiplam was available via a managed access agreement in the UK.

All patients are encouraged to continue to complete 6 monthly motor outcome measures and patient reported outcome measures. A questionnaire was developed via a online platform in which worldwide patients could provide more information and insight to help clinicians understand the SMA patients' views on use of Risdiplam, expectations, side effects and improvements reported.

67 patients/ parents of children, self-enrolled and completed the online survey approved by the local IRB. The survey link was shared with the SMA community via the SMA UK charity website, TREAT SMA and SMA social media groups to increase visibility and accessibility to the survey. The questionnaire could be access via a web link or QR code.
Patients / parents completed five sections looking at different aspects of the Risdiplam treatment journey: section 1: About you, Section 2: Your treatment choice, challenges and expectations, Section 3: Risdiplam treatment and outcomes, Section 4: Risdiplam clinic experience, Section 5: Risdiplam and fertility.

The questionnaire was completed by SMA patients and parents of children with SMA. Out of the 67 online responses, 45 were female and 22 male. Ages ranged: Below 18 years, n=12, whilst majority were age 18-54 years, n=48. Type 2 SMA was the largest respondent group n= 41 , Type 3 n=18 and Type 1 n=7. 82% of respondents were white Caucasian whilst 18% belonged to ethnic minorities. Of all 40% reported some type of TEAE and 55% didn't. 25% (17/67) of respondents reported diarrhoea, 24% reported headache, 10% had infections and 13% reported dermatological problems. SMA related health score post Risdiplam treatment was higher than at baseline score, (VAS mean Pre = 57.48 and Post Risdiplam = 65.42).

There were no new safety signals identified during the survey. There was a significant increase in the patient perceived health status post Risdiplam. More data is needed from a larger cohort of SMA patients.

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Bioequivalence and food effect assessment for a room-temperature stable risdiplam tablet formulation in healthy volunteers

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Risdiplam (EVRYSDI®) is an oral survival of motor neuron 2 pre-mRNA splicing modifier approved for the treatment of spinal muscular atrophy. The approved risdiplam dose for patients with a body weight ≥20 kg is 5 mg. The commercially available formulation is an oral solution that is drawn up in a syringe by the patient or their caregiver every day. A bioequivalent tablet formulation would provide patients weighing ≥20 kg with the option of replacing the current oral solution with the simpler daily administration of either swallowing a tablet or dispersing it in water and drinking the suspension.

Part 1 of this healthy volunteer study (NCT04718181) assessed the relative oral bioavailability of, and the effects of food and stomach pH on, two new risdiplam tablet formulations. Here we present the results from Part 2 of the study, which assessed the bioequivalence and food effect of the most promising tablet formulation from Part 1 versus the currently approved oral risdiplam solution. Using a four-way crossover design, each volunteer randomly received a single dose of 5 mg risdiplam oral solution in both fed and fasted states, and a single 5 mg risdiplam tablet (either swallowed whole or dispersed in water, depending on their group allocation) in both fed and fasted states.

The new risdiplam 5 mg tablet formulation was shown to be bioequivalent to the current commercially available oral solution of risdiplam, based on the pharmacokinetic parameters area under the concentration-time curve and maximum concentration. In addition, no relevant impact of food on the exposure of risdiplam for either the oral solution or the tablet was found.

The new risdiplam tablet formulation will provide a convenient alternative to the approved oral solution for patients taking the 5 mg dose of risdiplam. Additionally, administration of the oral solution or the tablet is not affected by whether individuals are in a fed or fasted state.

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Practical Use of Risdiplam (EVRYSDI®▼) in Individuals with Spinal Muscular Atrophy

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Risdiplam (EVRYSDI®) is a centrally and peripherally distributed, oral *SMN2* pre-mRNA splicing modifier that has been approved in the United Kingdom for the treatment of 5q spinal muscular atrophy (SMA) in patients two months of age and older, with a clinical diagnosis of SMA Type 1, Type 2 or Type 3 or with one to four *SMN2* copies.
Although risdiplam has a tolerable safety profile, its use has been associated with certain adverse events, which has been reported in clinical trials and the real world. Therefore, there has been an interest from the SMA clinical community for practical guidance on the management of those common adverse events. Three areas have been identified by neuromuscular experts as topics the SMA clinical community would particularly like guidance on. These include how to manage skin and gastrointestinal (GI) adverse events, and the impact of risdiplam on fertility.

In October 2022, a UK multi-disciplinary workshop, organised by Roche but led by specialist UK clinicians with expertise in managing individuals with SMA, was held to develop guidance for the clinical SMA community on the key considerations around fertility, and the management of GI and skin adverse events observed in individuals receiving or being considered for risdiplam.

Following a review of clinical trials, case studies and real-world evidence, the clinical expert panel provided recommendations on the management of GI and skin adverse events, and guidance around fertility.

The practical guidance developed by the specialists in this workshop is intended to provide helpful recommendations for the management of adverse reactions in individuals with SMA taking risdiplam and will be presented.

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Exploiting the drug repositioning strategy for the treatment of Spinal Muscular Atrophy: In vitro and in vivo results of a new effective SMN-dependent molecule

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Spinal Muscular Atrophy (SMA) is caused by the mutation of the survival motor neuron 1 (*Smn1*) gene. The consequent lack of SMN protein determines motor neuron (MN) impairment, skeletal muscle atrophy, and premature death. Fundamental limitations of current therapies still drive the need for new approaches aimed at increasing functional SMN production. Drug repositioning for SMA treatment represents a reliable tool to address significant unmet therapeutic needs.

A Drosophila-based screening identified GT5 (code name) as a promising therapeutic candidate for SMA.Then, the efficacy of GT5 was tested in vivo (delta7 mice, murine model of severe SMA) and in vitro (patient's iPSCs-derived-MNsand primary SMA fibroblasts and myoblasts). The SMN expression, the neuroprotective and anti-inflammatory effects have been evaluated by WB assays, immunofluorescence reactions and morphometric analyses. Moreover, we assessed the behavioral performance and survival in treated and untreated mice.

We observed that daily subcutaneous administration of GT5 in delta7 mice increased the SMN levels in the spinal cord (≥50%), quadriceps, and gastrocnemius (≥1 fold), compared to controls, also leading to improved motor skills. The analysis of the spinal cord ventral horns (lumbar tract) of GT5-treated mice confirmed: i) delayed MN degeneration (≤90%); ii) reduced levels of cleaved-caspase-3 (apoptotic marker) (≤63%), iii) lower neuroinflammation with reduced astrogliosis (GFAP signaling) (≤37%) and different degree of microglia ramification/activation compared with controls. Furthermore, GT5 mice skeletal muscles showed improved trophism and neuromuscular junction phenotypes. In vitro analyses also revealed that GT5 administration significantly prevented iPSC-derived MN degeneration and rescued the impaired formation of myotubes in an MN-myoblast co-culture.

Overall, these results support the GT5 repositioning for the SMA treatment and strengthen the value of this strategy for discovering new therapies for rare diseases.

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Identification of new SMN-independent compounds to counteract Spinal Muscular Atrophy: *In vitro* validation

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Spinal Muscular Atrophy (SMA) is the most common genetic cause of infant mortality. Due to the mutation/deletion of Survival Motor Neuron 1 (*Smn1*) gene and the consequent lack of SMN protein, SMA is characterized by the progressive degeneration of lower motor neurons (MNs). FDA and EMA have approved three revolutionary SMN-dependent treatments that are focused on increasing the production of the SMN protein. However, these approaches present some limitations: therefore, the identification of alternative/synergistic therapeutic strategies is strongly needed.

Thanks to a drug screening performed on a *Caenorhabditis elegans* SMA model, we identified new FDA-approved drugs able to rescue the neurodegeneration in the SMA-affected worms. The most effective molecules were then tested *in vitro* on primary cortical neurons from SMA delta7 mice (a severe SMA model) and on *Smn*-silenced NSC-34 cells (a MN cell line). Different analyses (MTT assay, neurolucida reconstructions, immunofluorescence reactions) were performed to assess cell viability and morphology. Moreover, to evaluate the drug efficacy and mechanism of action (MoA), live-cell analysis and WB were used to measure further morphological and functional parameters (including SMN expression, apoptosis and autophagy cascades, mitochondrial network/functionality).

Compared to untreated primary cortical cells, most of the compounds exerted positive effects, by significantly improving cell viability, increasing soma area and length/branching of neuronal processes, and influencing the synaptic vesicle distribution. On the contrary, gross morphological differences were not detected in treated vs untreated NSC-34 cells. By WB analysis, we also evaluated the SMN expression levels: since no differences were observed among groups, we suggest that the tested compounds act via a SMN-independent MoA. Preliminary results also show a modulation of the mitochondrial activity and of autophagic pathway, in both *in vitro* models.

Although additional analyses are necessary, our results suggest that the repositioned drugs identified are neuroprotective and could be combined with SMN-dependent treatments, to develop synergistic pharmacological strategies and improve the efficacy of the available ones.

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Identification of new SMN-independent compounds to counteract Spinal Muscular Atrophy: *In vivo* semi-automated drug screening in *C. elegans*

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The restoration of SMN protein levels is the basis of the three approved pharmacological therapies for SMA, which have brought incredible improvements to patients, but still present some limitations. Thus, it is largely accepted that a global therapeutic approach that includes all SMA phenotypes is still necessary, involving both SMN-dependent and SMN-independent strategies. To this aim we are identifying new small molecules that work in a SMN-independent way to be combined with actual treatments using an alternative animal model.

We took advantage of a *C. elegans* SMA model we developed, where *smn-1*, the *Smn1* ortholog, is specifically silenced in motoneurons (MNs), causing an age-dependent neurodegeneration. Using a semi-automated high content imaging system we performed an unbiased drug screening of an FDA-approved library consisting of more than 1200 compounds, With this approach we were able to analyse 384 compounds/week in triplicate on living animals.

We identified 4 new exciting leading compounds that counteract *smn-1* related neurodegeneration in *C. elegans*. Then, we validated the 4 compounds in a secondary screening and identified the dose-response curve. Finally, in collaboration with other groups, the hits were validated in vitro in mammalian systems.

Our results demonstrate that we can rapidly identify small molecules that suppress MNs degeneration by combining high content imaging of living animals with drug screening approaches. Interestingly, one of the compounds has been recently published to be effective in another SMA model in *C. elegans*, thus strongly supporting the efficacy our approach. We are now expanding the screening to other libraries and determining the time and the mechanisms of action of the hits. We think we have established a platform to deliver major progresses in defining new combinatorial treatments for preventing the neuronal death caused by *smn-1* loss in motoneurons.

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Use of a new long-term delivery method of antisense oligonucleotides to treat hiPSC derived motoneurons from Spinal Muscular Atrophy patients

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Spinal muscular atrophy (SMA) is the main neuromuscular autosomic recessive cause of infant mortality. The disease results from the deletion or mutation of the gene *Survival Motor Neuron 1* (SMN1), which causes the degeneration of the alfa motoneurons of the ventral horn of the spinal cord. Normally, healthy individuals have two copies of the SMN gene: SMN1 and SMN2. SMN1 produces the correct form of the protein while SMN2 has one base pair change in exon 7 that produces a defective form of the protein. Conversely, SMA patients do not have the SMN1 copy, so approximately 90% of the SMN protein that they can produce is defective.
In 2017 an antisense oligonucleotide (ASO) named Nusinersen was commercialized for the treatment of SMA patients. The mechanism of action of Nusinersen is to impede the splicing of exon 7 in the SMN2 copy, which will lead to the correct expression form of the protein. Currently, patients treated with Nusinersen need every four months intrathecal injections to deliver the ASO. The aim of the project is to establish a new long-term delivery mechanism for the introduction of this ASO.

We used first an *in vitro* model, motoneurons derived from hiPSC from control and SMA patients.

Because of this delivery method, specific free-uptake conditions were initially established, and we demonstrated that motoneurons were able to uptake the ASO. Moreover, we determined the optimal concentration and time-point for the motoneurons to uptake it. In the next step we made co-cultures of the motoneurons with the excipient from where the ASO is released, in order to check for biocompatibility and toxicity.

Currently, we are testing the release of the ASO from the excipient in the *in vitro* model, and will in the future test it using a model more near to the *in vivo* system as is the spinal cord organoids.

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Developing combinatorial therapies for the treatment of Spinal Muscular Atrophy

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Spinal Muscular Atrophy (SMA) is a neurodegenerative disease characterized by motor neuron loss, resulting in muscle weakness and paralysis. It is the leading genetic cause of infant mortality. Because even patients with non-fatal forms of SMA are considerably disabled, the identification of novel therapeutic strategies that slow or arrest the progression of the disease is urgently needed.
In 95% of cases, SMA is caused by mutations in the "survival of motor neuron 1" (SMN1) gene, which results in reduction in the levels of its translated product, the SMN protein. This leads to impairment of normal development/maintenance of the neuromuscular system and neuronal survival.

A therapeutic strategy that has recently advanced to the clinic is increasing SMN expression levels by using antisense oligonucleotides (ASOs). Although the use of ASOs represents an elegant therapeutic approach and an important advancement for the treatment of SMA, it does not seem to represent a complete "cure". Among others, an important limitation of ASOs is the amount of available SMN2 pre-mRNAs, particularly in individuals affected by the most common and severe SMA type 1, who have only one or two SMN2 copies, but likely require the highest and most rapid induction of SMN. Besides, the response to currently available treatments appears to be largely heterogeneous with early intervention emerging as the only predictive factor of symptom improvement in clinical practice

Here, we report the development of a peptide that can increase the amount of SMN protein within and beyond the nervous system. We show through a series of in vitro and in vivo analyses that boosting the levels of SMN during the initial phase of the disease, individually or in combination with an ASO, can significantly improve the impact of approved therapeutics towards SMA manifestations, thereby opening new perspectives for the treatment of this disease.

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High-throughput screening reveals candidate drugs for rescuing SMN-specific translational defects in SMA

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The Survival Motor Neuron (SMN) protein, deficiency of which causes Spinal Muscular Atrophy (SMA), has been proposed to be a ribosome-associated protein. SMN-primed ribosomes supervise the translation of a specific subset of mRNAs that are enriched in enhancer regulatory sequences at the 5'UTR and in rare codons at the beginning of the coding sequence. In SMA, decreased levels of SMN protein lead to an impairment in protein synthesis that can be rescued by antisense oligonucleotides that restore SMN expression. Despite the approval of three SMN-dependent therapies, SMA still remains a disease that has no cure yet. Here, our objective is to explore novel SMN-independent treatments that could be used to support already approved therapies with the overarching aim of using them as complementary therapeutical approaches.

Exploiting a cell line model of SMA and a dual luciferase sensor of SMN-specific translation, we performed an automated high-throughput screening and tested a library of small molecules for drug repurposing with the goal of rescuing SMN-specific defective translation. To validate our hits, we performed polysome profiling and metabolic labelling in multiple cell lines with various levels of SMN expression as models of severe and mild SMA.

We implemented the high-throughput screening employing a construct containing enhancer regulatory sequences in the 5'UTR and rare codons at the beginning of the coding sequence to mimic SMN-specific transcripts and to act as a translation sensor in the SMA-like cell line.
First, we optimized the protocol for the screening by adjusting the number of cells, the transfection conditions, and the kit to monitor the translation efficiency of the reporter. We built a 4-day protocol consisting of cell seeding (0h), plasmid transfection (24h), drug administration (48h), and luciferase reading (72h).
Next, we selected a library with more than one hundred inhibitors of kinases and phosphatases and identified compounds that specifically enhance the translation of the sensor. Four interesting candidates emerged among all the tested molecules.
Finally, we performed luciferase assays, polysome profiling, and SUnSET assays, to validate our screening results in different cell lines that present different SMN expression levels and recapitulate different disease subtypes. We demonstrated that, upon drug administration, translational defects in the SMA-like cell lines are rescued.

In this work, we identified candidate drugs that rescue SMN-specific defects in translational and that open up undiscovered scenarios for the investigation of novel SMN-independent therapies.

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Experience with Combination treatment of Risdiplam and Gene Therapy in a Child with Spinal Muscular Atrophy in Bangladesh: A Case Report

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Over the years, significant advancements have been made in the treatment of spinal muscular atrophy (SMA), particularly with the introduction of therapies such as oral SMN splicing therapy (risdiplam) and gene therapy. Risdiplam has shown promising results in increasing the production of functional survival motor neuron protein (SMN) in SMA patients and gene therapy by delivering a functional copy of the SMN1. This case report presents the experience of a combination treatment approach involving risdiplam and gene therapy in a child diagnosed with SMA in Bangladesh, providing valuable insights into the clinical management and outcomes of this treatment strategy.

A 12-month-old boy 2nd-issue of nonconsanguineous parents came to the Paediatric Neurology OPD, NINS&H with complaints of motor delay and repeated attacks of respiratory tract infections from four months of age. On examination, he was alert, had partial neck control, generalized hypotonia with absent reflexes and no mobility. His NCS & EMG showed anterior horn cell disease. He was first diagnosed as having cystic fibrosis, though he had no such symptom, except for repeated respiratory tract infections. At 13 months he was diagnosed with SMA type1 through genetic testing (MLPA), with 0 copy of exon 7 in SMN1 gene and 2 copies of SMN2. After consulting a geneticist and pulmonologist he was given targeted therapy, first with oral risdiplam at the age 16 months, then onasemnogene abeparvovec (Zolgensma-1.1 × 1014 vg/kg) in combination with risdiplam at 22 months. The patient tolerated both therapies well, with only minor, transient side effects. The child's progress was closely monitored through clinical assessments, including motor function evaluations, respiratory function tests and quality of life assessments. After taking risdiplam, the patient's motor function improved, with increased muscle strength and mobility, whilst no respiratory problems developed. After gene therapy no further deterioration was observed during the follow-up period.

Data on combination treatment are limited. This case report highlights the promising results of this combination treatment approach involving risdiplam and a gene therapy in a child with SMA and sheds light on its potential benefits. These findings are consistent with the FIREFISH, SUNFISH and STR1VE trials, with clinically meaningful motor function improvements in SMA Type 1 patients. The findings from these studies, combined with the results of this case report, support the rationale for using a combination treatment approach to maximize therapeutic benefits in SMA patients. The precise mechanisms underlying the synergistic effects of risdiplam and gene therapy in SMA management warrant further investigation. It is possible that the combination of risdiplam-induced SMN protein production and gene therapy's genetic correction led to a stronger therapeutic effect, resulting in improvements in motor and respiratory function. Here, combination treatment was well-tolerated without any significant adverse events. However, long-term follow-up and larger-scale studies are needed to further assess safety and durability. In conclusion, the combination treatment of risdiplam and gene therapy in a child with SMA in Bangladesh demonstrated promising results in terms of motor function and respiratory improvement. However, further research is needed to confirm these findings.

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'A small step for a better tomorrow' Journey of a Spinal muscular Atrophy Type 1 patient with RNA splicing modifier- oral Risdiplam for the 1st time in Bangladesh

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Spinal Muscular Atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by mutations in the Survival Motor Neuron 1 (SMN1) gene, which is responsible for producing survival motor neuron (SMN) protein. Without sufficient levels of SMN, motor neurons of the spinal cord gradually degenerate, leading to muscle weakness and atrophy. Type 1 SMA is the most severe form and typically appears in infancy. Babies with Type 1 SMA often have severe muscle weakness, difficulty breathing, and may not survive beyond a few years without intensive medical support. Risdiplam is a selective SMN2 RNA splicing modifier which promotes production of full-length SMN2 mRNA and functional SMN protein. Here we present the one year journey of a 9 months old SMA type 1 patient who got treatment for SMA type 1 for the 1st time in Bangladesh.

The 1st dose of oral Risdiplam at the dose of 0.2 mg/kg/day was given on 20th March, 2022 at Paediatric Neurosciences Department of BSH&I. Since then the child got her everyday calculated dose and was closely monitored in a multidisciplinary approach for her overall growth, development and complications.

Results were measured using the Hammersmith Infant Neurological Examination (HINE), which assesses 8 developmental milestones for infants, including head control, sitting, voluntary grasp, ability to kick, rolling, crawling, standing, and walking. Optimal scores at 9 and 12 months are at least 73 in a developmentally age appropriate child. The starting score was only 14 for the patient it dramatically improved to 48 at the age of 2 years.

Oral Risdiplam can be life saving for the treatment of SMA type 1 patient if started as early as possible. It also improves the motor function and quality of life for the patient.

Key words
SMA Type 1, Risdiplam

Beyond the Motor Neuron

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Mild alteration in cardiac parameters correlate with a disruption in PPARγ signaling in a moderate mouse model of SMA

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Although primarily a motor neuron disease, in SMA, defects in other non-neuronal tissues are increasely described in both patients and mouse models. This includes defects within the heart, comprising structural and functional perturbations which manifest to varying severities in patients and mouse models. The *Smn*$^{2B/-}$ mouse model offers an opportunity to understand the manifetation of pathology over a protracted time course and gain insight into the underlying mechanisms of pathology.

Here we aim to perform a detailed structural, functional and molecular study of the hearts in the intermediate *Smn*$^{2B/-}$ mouse models of SMA and identify molecular signatures which unite mouse models.

We have combined histopathological analysis, high resolution electrocardiographic imaging and tandem mass tagging proteomics to characterize the hearts of *Smn*$^{2B/-}$ mice over the disease time course, and perform comparative analysis with other mouse models to identify consistent changes which can contribute to heart pathology in SMA.

Here we show that the hearts of the *Smn*$^{2B/-}$ mice display a thinning of the ventriclar walls which is consistent with defects previously described in more severe mouse models of SMA. However the majority of structural changes within the hearts of *Smn*$^{2B/-}$ mice are mitigated by accounting for the smaller body size of the SMA mouse model. A detailed electrocardiophy study revealed a presevation in distolic and systolic function but changes in longituinal strain which are indicative of early signs of cardiac stress. We have used tandem mass tagging proteomics to perform a longitudinal study of the proteome of the hearts of *Smn*$^{2B/-}$ mice and reveal a progressive dysregulation of oxidative phorylation and fatty acid metabolism. We further show consistent perturbations in these pathways in 3 mouse models of SMA on the day of birth. We further identify PPARγ signalling as a upstream regulator of the identified dysregualted pathways.

Together this work indicates that although structural changes in the heart can be overstated when methods fail to account for body size, there are functional defects which can predispose the heart to subsequent failure. We identify a common molecular signature across mouse models and througout disease duration pointing to a dysregulation in oxydative phosphylaton and fatty acid metabolism, and suggest that manipulation of PPARγ signalling offers an opportunity to impact upon these pathways.

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β-adrenergic signaling is impaired in cardiomyocytes in a mouse model of spinal muscular atrophy

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Spinal muscular atrophy (SMA) is a genetic degenerative disease caused by mutations in the *SMN1* gene that encodes the survival motor neuron (SMN) protein. Reduced SMN protein, a critical component of the spliceosome, results in loss of motor neurons in the anterior horn of the spinal cord and consequent muscle atrophy. While the effects of SMN deficiency on motor neuron function and survival is well-studied, the effects on extraneuronal tissues are still emerging. Cardiac muscle pathologies, including structural defects, tachycardia, and cardiomyopathy, have been observed in both SMA patients and mouse models of the disease. We have previously shown that intracellular Ca^{2+} cycling and contraction dynamics are altered in SMAΔ7 mice that replicate many of the key features of SMA. One hypothesis for the cause of these pathologies is loss of sympathetic neurons. Alternatively, a lack of β-adrenergic sensitivity would cause a similar phenotype. To investigate this, we utilized isolated cardiomyocytes from SMAΔ7 mice to determine the effect of β-adrenergic stimulation with isoproterenol on cardiac intracellular Ca^{2+} transients. We show that β-adrenergic stimulation with isoproterenol has no effect on intracellular Ca^{2+} transients in SMA mice. This effect is not due to a change in β-adrenergic receptor expression. In addition, we utilize electrocardiographic analysis in awake mice to determine the *in vivo* effect of β-adrenergic stimulation with isoproterenol. These findings provide novel insight into potentially disease modifying aspects of SMA cardiac pathology.

P45

Melatonin ameliorates neuronal and peripheral pathologies in *Smn$^{2B/-}$* SMA mice

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Going beyond neuronal pathologies in spinal muscular atrophy (SMA), developmental and metabolic pathologies in many peripheral tissues, including muscle, liver, pancreas, and adipose tissues, have been found in both SMA mouse models and SMA patients. Therefore, peripherally targeted therapies for SMA that can complement existing gene-based therapies are at the forefront of second-generation therapies. To identify commercially available drugs predicted to ameliorate peripheral pathologies in SMA, we performed RNA sequencing on symptomatic Taiwanese *Smn$^{-/-}$;SMN2* mice and WT animals. The KEGG-DRUG database, iPathwayGuide, and Drug-Gene Interaction database were used to identify commercially available drugs predicted to restore the levels of differentially expressed genes in SMA muscle. A strong pharmacological candidate identified by this combined bioinformatics and drug repurposing approach is melatonin, an FDA-approved nutraceutical, normally used for sleep disorders.

The potential therapeutic impact of melatonin on SMA pathology has been assessed in both *in vivo* (C2C12s mouse muscle cell line) and *in vitro* (severe Taiwanese *Smn$^{-/-}$;SMN2* mice and milder *Smn$^{2B/-}$* mice). In an analysis of molecular effects, tissues harvested from *Smn$^{2B/-}$* mice treated with 50 mg/kg/day melatonin were compared with untreated *Smn$^{2B/-}$* littermates. This investigation specifically focused on the predicted target genes *Per1, Bcl2, Sirt1*, and *Ror- α*, which were previously identified in combined in silico analysis. Further insights were pursued through Nissl and Oil-Red-O staining were utilized to explore melatonin's impact on alpha motor neuron loss in the spinal cord and lipid accumulation in the liver, respectively.

Our *in silico* analyses predicted that the expression of *Per1, Bcl2, Sirt1* and *Ror- α* genes is increased in SMA skeletal muscle and can be downregulated by melatonin treatment. First, we validated the abnormal expression of *Per1, Bcl2* and *Sirt1* in symptomatic skeletal muscle tissue in both SMA mouse models. Subsequent research has stated the safety and pharmacological impact of melatonin on the C2C12s cell line, uncovering a dose-dependent alteration in the expression levels of the target genes. The potential molecular impacts of a 50 mg/kg/day melatonin treatment were assessed in relation to the expression levels of key target genes in milder *Smn$^{2B/-}$* mice. Post-treatment, both the symptomatic skeletal muscle and white adipose tissue displayed elevated expression of all these genes compared to untreated counterparts. In the symptomatic liver tissue, all genes, except for *Sirt1*, experienced increased expression after 50 mg/kg/day melatonin application. On the other hand, the spinal cord tissue presented a decline in *Per1* mRNA levels, while the other three genes remained unaffected. From a metabolic standpoint, there was an apparent enhancement in the mitochondrial -based oxidative capacity, indicated by the elevated expression of *Cat* and *Sirt1* in muscle, liver, and white adipose tissue. Notably, the rise in *Glut4* expression, observed solely in skeletal muscle tissue, suggests melatonin's potential role in mitigating insulin resistance. When evaluating the histopathological impacts of the treatment on SMA pathology, there were no observable changes in the number of alpha motor neurons and lipid accumulation within the liver. Conclusively, the administration of 50 mg/kg/day melatonin on a less severe SMA mouse variant led to notable improvements in weight and survival outcomes of milder *Smn$^{2B/-}$* mice.

Melatonin holds the potential to ameliorate the observed disruptions in glucose, lipid, and circadian rhythm metabolisms seen in SMA patients and mouse models. It is presumed to do so by modulating mitochondrial functions (through *Sirt1* and *Cat*), mitigating insulin resistance (via *Glut4*), and regulating apoptosis (via *Bcl-2*). Consequently, our integrated strategy of bioinformatics and drug repurposing stands out as a potent method for foreseeing novel, second-generation treatments for the muscular and metabolic abnormalities present in SMA.

Acknowledgements

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P46

A Spine full of Stars – Spinal Astrocytes in late-onset SMA Pathology and Therapy

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Spinal Muscular Atrophy (SMA) is a genetic neuromuscular disorder caused by a defect in the *survival of motor neuron* (SMN) *1* gene, resulting in progressive loss of spinal motor neurons (MNs) and muscle weakness. Nowadays, SMN-enhancing drugs are highly effective when administrated early. However, even if these drugs are highly effective when administrated early, not all SMA patients benefit sufficiently. Some remain non-responders, such as adult patients suffering from late-onset SMA who have undergone a long-standing irreversible loss of MNs before starting their therapy. In these patients, a solely SMN restore has the ability to stop the progression of SMA, while motor functionality can only be slightly restored. Therefore, to improve the disease stage of patients who have not benefited from the current SMA therapies, identifying other targets to develop supporting strategies for current SMN-enhancing drugs is indispensable. To identify such targets, a greater understanding of cellular pathomechanisms beyond MN loss is of immense relevance.
Our recent work suggests a potential role for spinal astrocytes in the pathogenesis of late-onset SMA and identified these cells as an early driving force for MN loss.

Different *in vivo* and *in vitro* models, including a mouse model of late-onset SMA subforms, cell and tissue cultures, patient-based induced astrocytes, and liquor samples. Those models have been evaluated by immunostaining, Western Blot and qPCR analysis, electrophysiology, behavioral tests, and *in silico* analysis.

In late-onset SMA, spinal astrocytes are active before the first loss of spinal MNs. Besides this early activation, astrocytes show alterations in the expression and function of proteins essential for MN physiology, such as the excitatory amino acid transporter (EAAT) 1 or 2 and the inward rectifier potassium channel 4.1 (Kir4.1).
While Kir4.1 downregulation leads to increased expression and release of brain-derived neurotrophic factor (BDNF) by MEK/ERK signaling, resulting in the upregulation of calcium-permeable AMPA-receptors on spinal MNs, making MNs more vulnerable to glutamate, the reduction of EAATs increases the extracellular glutamate-level in the spinal cord and contribute to the degeneration of spinal MNs by excitotoxicity. When EAAT expression was increased using arudic acid, MN loss was prevented.
Both described astrocyte-based pathomechanisms revealed multiple potential therapeutic targets that already approved drugs could address.

Our data demonstrate the crucial role of spinal astrocytes in the pathogenesis of late-onset SMA. Furthermore, their therapeutic potential is revealed. This makes astrocytes an exciting target for complementing current SMN-enhancing strategies.

P47

Network biology-based analysis of SMA: Identification of disease relevant protein targets and altered signaling in severe and mild SMA mice

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Strategies for increasing SMN expression levels have been a ground-breaking step for the treatment of Spinal Muscular Atrophy (SMA). However, SMN is involved in several molecular and cellular mechanisms and the identification of disease-relevant processes is challenging. This complexity of SMA results in the need for a network-biology analysis approach. In this project, we aim to (1) identify expressional and phosphorylation changes in spinal cord samples at disease onset in different SMA mouse models (Taiwanese, $Smn^{2B/-}$) and (2) analyze signaling alterations and kinase phosphosite associations of significantly dysregulated proteins. To combine the current knowledge about SMN and the mechanisms altered in SMA, network analysis was used. We additionally used SMN-interactome data sets to mechanistically elucidate the molecular link to SMN in the network of dysregulated targets. This network highlights hubs and bottleneck molecules at critical positions.

To better understand the central pathways of SMA, we performed quantitative (phospho) proteomic analyses of L1-5 spinal cord from the severe Taiwanese and the mild $Smn^{2B/-}$ mouse model at a presymptomatic stage (P3 and P12-13, respectively).

We identified 330 and 220, respectively, dysregulated proteins with an overlap of 32 proteins and several upstream regulators. Those dysregulated proteins and regulators include already described proteins in SMA, as e.g., Small nuclear ribonucleoproteins, neurofilament proteins and mTOR or MAPT. Both data sets include targets involved in splicing, cytoskeletal proteins, translation, and DNA replication (severe model only). Therefore, the data reflect SMA at different disease stages. Phosphoproteome analyses of both models revealed changes in signaling pathways and kinase families associated with dysregulated phospho-sites.

This study combines different molecular levels and methods to describe SMA on a systems level and enables the interpretation of single protein changes in the disease context.

P48

Central and peripheral delivery of ASO 10-27 increases SMN levels in spinal cord and peripheral tissues preserving neuromuscular junction and lymphoid organs pathology in SMNΔ7 mice

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Besides motor neuron dysfunction and loss, neuromuscular junction (NMJ) and skeletal muscle defects are key hallmarks of SMA. Moreover, data indicate the contribution of additional peripheral organs alterations to the severity of disease. The antisense oligonucleotide (ASO) nusinersen can restore the SMN deficit found in SMA and dramatically improve its associated neuromuscular dysfunction. However, the impact of SMN restoration in preventing multiorgan alteration and ameliorating SMA pathology needs to be clarified. In addition, the ASO delivery route could have a differential impact on the improvement of SMA peripheral organ pathology. The aim of this study is to analyze the effect of the nusinersen-like ASO 10-27, administered via different routes, in the SMA alterations found in non-central nervous system (CNS) tissues such as skeletal muscle and lymphoid organs.

Wild-type and SMNΔ7 mutant mice were treated with intracerebroventricular (ICV) and/or subcutaneous (SC) injections of either ASO 10-27 or vehicle solution. The ICV treatments were performed at P0 (6.5 mg/kg of ASO, single injection), whereas SC administration was carried out at P1 and P3 (50 mg/g/day of ASO). Weight, survival, and motor phenotype of mice were determined. Histological, immunocytochemical and western blot analysis were performed in tissue samples from skeletal muscle, thymus and spleen at P14.

ICV treatment with ASO 10-27 prolonged lifespan, improved motor behavior and increased SMN levels in the CNS of SMNΔ7 mutant mice. Surprisingly, a partial restoration of this protein was also observed in peripheral organs. NMJs of SMA mice exhibited denervation, reduced size, and delayed maturation. SMA was also accompanied by defects in lymphoid organs. Compared to controls, spleen of SMA mice showed a decrease in cell density in both the white and red pulp, and in the number of B lymphocytes. Cell density in SMA thymus was reduced in cortex but augmented in medulla, which exhibited higher T lymphocyte numbers. All these alterations were prevented by the ICV treatment with the ASO. SC injections also restored SMN levels in the peripheral organs, especially in muscle, and surprisingly, although in a lesser extent, in the spinal cord.

Therapeutic restoration of SMN protein is needed in both CNS and peripheral tissues due to their independent contributions to SMA pathology. ICV administration of ASO is able to partially restore SMN levels in peripheral organs. This could be explained by some dissemination of the agent due to the immature state of the blood-brain barrier. SC injections promoted higher SMN restoration at the peripheral system. Thus, new approaches combining CNS and peripheral administration of ASO 10-27 may maximize its positive clinical effect in SMA.

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P49

Correlation of individual SMN protein with observed efficacy in the risdiplam clinical trials

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Risdiplam (EVRYSDI®) is a centrally and peripherally distributed, oral survival of motor neuron 2 (*SMN2*) pre-mRNA splicing modifier approved for the treatment of spinal muscular atrophy (SMA) in 100 countries worldwide.

The safety and efficacy of risdiplam were assessed in clinical studies of patients with SMA, from infants to adults, with a body weight ≤109 kg and ≤61 years of age. Pharmacokinetic and pharmacodynamic data were used to select the dosing regimen of 0.2 mg/kg for infants aged between 2 months and 2 years, 0.25 mg/kg for children ≥2 years of age with a body weight <20 kg, and 5 mg for patients with a body weight ≥20 kg.
The pharmacodynamic markers *SMN2* mRNA and SMN protein were assessed in blood samples obtained from all patients over the duration of the study for up to 2 years.

Risdiplam's mode of action was confirmed by the shift from *SMN2Δ7* mRNA to full-length mRNA. Risdiplam treatment led to a ≥2-fold median increase in SMN protein levels within 4 weeks of the start of treatment, which was maintained throughout treatment.
Exposure–response analyses were conducted to assess the correlation between the individual SMN protein level and the observed efficacy in the pivotal studies in patients with Type 1 (FIREFISH Part 2; NCT02913482) and Types 2/3 SMA (SUNFISH Part 2; NCT02908685). The analyses to assess the correlation with efficacy were conducted with the absolute SMN protein values during the study treatment period, and also with the change from baseline in SMN protein level obtained for each patient pre- and post-risdiplam treatment. The results of the correlation of the SMN protein in blood versus the assessed efficacy endpoints (FIREFISH: sitting, head control and Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; SUNFISH: 32-item Motor Function Measure and Revised Upper Limb Module) will be presented for both studies.

P50

Dopaminergic system role in a *C. elegans* model of SMA

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SMA has been considered a motor neuron specific disorder, but this selectivity is in contrast with the fact that *Smn1* is a ubiquitous gene with housekeeping functions. Indeed, recently SMA has been re-defined as a multi-system disorder. In iPSCs-derived neurons from SMA patients, in mice and in *C. elegans* models of SMA, genes of the dopaminergic pathway resulted to be dysregulated at the transcriptional and post-transcriptional level.

We took advantage of multiple *C. elegans* SMA models mutated in *smn-1,* the *C. elegans* ortholog of *Smn1,* to investigate by HPLC, formaldehyde induced fluorescence (FIF), behavioral tests and genetics, the unexplored connection between SMA and the dopaminergic (DA) system *in vivo*.

We performed DA quantification in total extracts and *in vivo* (by detecting with FIF the DA related-fluorescence in dopaminergic neurons), and revealed a reduction in total and in intracellular dopamine. A DA-related behavior in *C. elegans* SMA models was found impaired, suggesting that the reduction of dopamine causes an alteration in the capacity to recognize the presence of food. We also confirmed the reduction in intracellular dopamine and in recognition of food in animals silenced for *smn-1* only in dopaminergic neurons, suggesting a cell-autonomous role. Since *bas-1*/AADC(*Aromatic L-amino acid decarboxylase)* expression has been found reduced in several mutant models of SMA, we overexpressed *bas-1* in dopaminergic neurons and partially rescued the behavior defect. Further, the administration of the DA precursor L-DOPA was able to partially rescue the reduction observed in intracellular DA and the behavioral defect. Interestingly, the overexpression of *bas-1* was also able to rescue a SMA-related defect, by ameliorating the locomotion impairment showed by *C. elegans* SMA models.

Taken together our results point out to a dysfunction of the dopaminergic system in a *C. elegans* model of SMA that, if confirmed in patients, may account for mood alterations observed in some SMA patients. Most importantly, our results may suggest new pharmacological combinatorial approaches to reduce the effects of *Smn1* loss in dopaminergic neurons of SMA patients. Interestingly, we also demonstrated, for the first time, the possibility to ameliorate SMA-specific locomotion defects by modulating a dopaminergic gene, like *bas-1*/AADC.

P51

Liver SMN restoration rescues *Smn$^{2B/-}$* mouse model of SMA: The key to rescue whole-body pathology?

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Spinal muscular atrophy is a childhood neuromuscular disorder characterised by alpha motor neuron loss and muscle atrophy. It is an autosomal recessive genetic disorder caused by loss of survival motor neuron gene 1 (*SMN1*), which leads to a depletion in survival motor neuron (SMN) protein. While SMA is primarily identified as a motor neuron disease, the restricted expression of SMN in motor neurons is not enough to rescue the SMA phenotype. Our previous findings demonstrate an increased susceptibility to developing dyslipidaemia and liver steatosis in SMA. Fatty acid metabolism defects and elevated triglycerides resembling non-alcoholic fatty liver disease (NAFLD) are present in *Smn$^{2B/-}$* SMA mice. A systemic approach to SMA therapy may improve therapeutic outcomes of SMA patients. We aim to determine whether fatty liver is an intrinsic or extrinsic impact of SMN depletion in *Smn$^{2B/-}$* mice using an AAV9 liver specific promoter delivering SMN.

Weight, motor function and survival of the *Smn$^{2B/-}$* mouse model have been conducted. We have performed western blot analysis to determine SMN expression levels in various CNS and peripheral tissues. We have also determined myofiber area size and cell fate balance within pancreatic islets using immunohistochemistry techniques.

We demonstrate that AAV9-albumin-*SMN* successfully expresses SMN in the liver but not in the central nervous system (CNS) and other peripheral tissues in P9 pups. We have found liver intrinsic rescue of SMN, alone, is sufficient to significantly increase survival of *Smn$^{2B/-}$* mice. Interestingly, we see a rescue of pancreas pathology but not a rescue of muscle myofiber area. Our results suggest the fatty liver phenotype in *Smn$^{2B/-}$* mice is a direct impact of liver intrinsic SMN loss.

We conclude that liver SMN is vital during development. We speculate that SMA is a developmental disorder, whereby different organs require varied levels of SMN throughout development. This work provides insight into the contribution of liver pathology in SMA and will improve our understanding of SMA as a multisystem disorder.

P52

Cerebellar circuit dysfunction in a mouse model of severe spinal muscular atrophy

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Spinal muscular atrophy (SMA) is characterized by the degeneration of spinal motor circuits resulting in impaired voluntary movement and muscle atrophy. Whether other regions of the central nervous system, e.g. brain, are affected in SMA is largely unknown. Cerebellar motor circuits are important for voluntary movements by processing proprioceptive information to modulate motor output, both of which are affected in SMA. Previously, we and others found morphological alterations of the cerebellum in SMA mice and patients. These include selective degeneration of Purkinje cells and excitatory synapses of the parallel fibers onto Purkinje cells which originate from the granule cells. These Granule cells receive excitatory inputs from the mossy fibers. In contrast to the morphological characterization, the function of the cerebellar circuitry in SMA is barely understood.

We applied whole-cell patch-clamp recordings to investigate the active and passive properties of Purkinje cells and granule cells of end-stage SMNΔ7 mice. To investigate synaptic transmission onto Purkinje cells and granule cells, we stimulated parallel and mossy fibers, respectively.

Whole-cell patch-clamp recordings revealed a decrease of capacitance and increase of membrane resistance of Purkinje cells, indicating a decreased cell size in SMNΔ7 mice. SMA Purkinje cells exhibited hyperexcitability and broaden action potentials which resulted in a decreased spontaneous and evoked firing frequency. This indicates that the overall output of Purkinje cells is impaired in SMA mice. Recording of Purkinje cells following stimulation of excitatory parallel fibers deriving from granule cells showed an increased facilitation of excitatory synapses, demonstrating weakened excitatory synaptic transmission between granule cells and Purkinje cells. Recordings directly from granule cells revealed hyperexcitability and broadened action potentials that resulted in decreased firing frequency.

In summary, SMNΔ7 mice exhibit severe functional impairments of the excitatory cerebellar motor circuits resulting in a decreased output of Purkinje cells. This suggests a dysfunctional aspect of the cerebellum which might contribute to the overall SMA phenotype.

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P53

Peripheral organs defects in spinal muscular atrophy: Actin dysregulation

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Motoneurons have long been the primary target in identifying mechanisms of SMA and developing treatments. Initial investigations have unveiled a crucial role for actin cytoskeleton dysregulation in the pathogenesis of SMA in neuronal tissues. The Rho-kinase (ROCK) pathway has previously been linked with this dysregulation of actin and differential phosphorylation of downstream effectors of two major actin associated proteins, profilin2a and cofilin. However, given the ubiquitous expression of SMN, it is paramount to identify abnormalities in peripheral organs. In this study, we analyze actin dysregulation as a pathogenic mechanism in peripheral tissues in SMA models.

We conducted a comprehensive analysis of peripheral organs from different post-natal timepoints using the Taiwanese and 2B- mouse models. We aimed to elucidate the expression and phosphorylation levels of two critical proteins, profilin and cofilin. To investigate alterations in the signalling of these proteins, we employed a transcriptomic approach to analyze these dysregulations and their upstream targets further. Specifically, we used Oxford Nanopore RNA sequencing to examine the gene expression profiles in both the kidney and heart of the Taiwanese mouse model at onset of symptoms.

The findings from our study displayed considerable variations across different organs. In kidney, there was a notable downregulation in the expression of both profilin and cofilin. Intriguingly, at the onset of symptoms, profilin exhibited a state of hyperphosphorylation. In contrast, in the heart, the expression levels of both profilin and cofilin did not exhibit significant changes. However, the signalling of profilin was notably affected at the early symptomatic timepoint. Moreover, our transcriptomic analysis revealed a significant impact of both organs in the Taiwanese mouse model at onset of symptoms.

Our study has broadened the understanding of the molecular intricacies underlying actin dysregulation in peripheral organs of SMA. The observed variations in protein expression and phosphorylation are indicative of a highly dynamic process that is differentially dysregulated across several organs, highlighting the complexity of SMA's systemic effects. Furthermore, the transcriptomic approach allowed for a more comprehensive insight into understanding the role of the actin cytoskeleton in SMA pathogenesis in vital organs. This broader perspective holds the potential to enhance treatment strategies and improve the overall well-being of SMA patients.

P54

Temporal analysis of neuronal and peripheral features of SMA in the *Smn$^{2B/-}$ mouse model*

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Spinal muscular atrophy (SMA) has been classically regarded as a motor neuron disorder due to the preferential vulnerability of motor neurons to the loss of survival motor neuron (SMN) protein. However, a wide range of non-neuronal tissues are affected in SMA patients and animal models of the disease. The *Smn$^{2B/-}$* mouse model demonstrates progressive motor weakness and motor unit pathology as well as abnormalities in peripheral tissues, allowing for closer examination of the non-neuronal contributions to SMA disease. This model displays severe metabolic defects, including liver and pancreatic abnormalities that are similar to symptoms observed in SMA patients. However, the mechanisms of these defects and their relationship to motor unit pathology throughout development is not clear.

We used the *Smn$^{2B/-}$* mouse on a C57BL/6 background, a model that demonstrates peripheral abnormalities, to conduct an in-depth temporal analysis of both neuronal and non-neuronal SMA characteristics throughout development. Mice were collected at several timepoints from P3 to P19 and characterized for motor neuron loss, plasma neurofilament light chain (NfL) levels, neuromuscular junction pathology, muscle atrophy, liver pathology, pancreas pathology and glucose metabolism defects.

Liver steatosis was first apparent at P13, while muscle fiber size was reduced beginning at P15. Plasma NfL levels were elevated beginning at P11 and increased with the progressing severity of disease in this model. Further experiments are being performed to determine the onset of the remaining SMA characteristics.

The results from this study will provide an in-depth temporal characterization of the *Smn$^{2B/-}$* mouse model of SMA. Understanding the onset of each feature over time will also reveal potential interactions between tissues during disease course and their individual contributions to overall disease. With the increasing use of life prolonging SMA treatments, it is important to understand the independent contributions of peripheral tissues to disease, as these features may become more prominent in an aging SMA population.

P55

Exploring the role of microRNA125b-5p in regulating microglial mediated neuroinflammation in Spinal Muscular Atrophy

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Spinal Muscular Atrophy (SMA) is a neuromuscular disorder associated with mutations in the survival motor neuron 1 (*SMN1*) gene. The intricate molecular mechanisms underlying motor neuron degeneration in SMA remain elusive, primarily due to the widespread expression of the SMN protein and its involvement in various cellular processes. This complexity presents a unique opportunity to identify various novel therapeutic targets, fostering innovative approaches in SMA research and treatment development. Recent investigations have highlighted the significance of microRNAs in fine-tuning various molecular processes, including microglial-mediated neuroinflammation. Among these, microRNA-125b-5p stands out as a microglial-enriched microRNA known to target the TNFAIP3, a crucial regulator of the NFKß1 pathway. Our study aims to explore the impact of microRNA-125b-5p on the TNFAIP3/NFKß1/TNF-α neuroinflammation pathway in SMA while highlighting the pivotal role of microRNAs in regulating microglia-mediated neuroinflammation.

We employed previously isolated spinal cord tissues from two distinct groups of mice: the severe SMA (*hSMN2*)2+/-; Smn-/- and Heterozygous unaffected (*hSMN2*)2+/-; Smn+/- Taiwanese mice. New-born SMA mice at postnatal day 0 (PND 0) were treated with a single subcutaneous injection of a 25-mer morpholino antisense oligomer (PMO25) at 40µg/g targeting *hSMN2* exon-7 to promote exon inclusion. Spinal cord tissues were collected from SMA untreated, SMA treated with PMO25, and heterozygous littermate control mice, at PND 7. Using real-time quantitative PCR, we measured changes in expression of h*SMN2-FL*, *Tnfaip3*, and microRNA-125b-5p transcripts in different groups of mice. We also measured SMN and TNFAIP3 protein expression using western blot analysis.

In the SMA mice, there was a notable increase in the expression levels of microRNA-125b-5p. Conversely, when these mice were treated with PMO25, there was a significant reduction in microRNA-125b-5p levels. This reduction in microRNA-125b-5p coincided with an increase in the expression of *SMN2-FL*, as observed at both the RNA and protein levels. Importantly, no discernible differences were detected in *Tnfaip3* transcript levels across the various groups of mice.

Elevated levels of microRNA-125b-5p in SMA mice highlight a significant connection between SMN and this microRNA. With this association established, our next objective is to confirm the post-transcriptional effects of microRNA-125b-5p by assessing TNFAIP3 protein levels. Additionally, our ongoing research aims to validate the potential of modulating microRNA-125b-5p expression to mitigate NFKß1-mediated neuroinflammation. To achieve this, we are currently in the process of developing a mouse microglial cell line with reduced SMN expression. This model will enable us to directly link microRNA-125b-5p levels with changes in neuroinflammatory markers regulated by the NFKß1 pathway.

P56

Impaired renal clearance and tubular function in patients with spinal muscular atrophy

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Homozygous loss-of-function of the *survival motor neuron 1 (SMN1)* gene results in deficiency of the ubiquitously expressed SMN protein, which is the cause of spinal muscular atrophy (SMA). Although alpha-motor neurons are most vulnerable to SMN deficiency, this may also cause abnormal anatomy or function of other tissues and organs. The adult human kidney expresses SMN levels similar to those found in the spinal cord. The aim of this study was therefore to assess renal function in patients with SMA in detail.

We performed a combined cross-sectional and longitudinal study in patients participating in an ongoing observational cohort study on SMA in the Netherlands. Baseline data were collected before the start of SMN-augmenting therapy and during follow-up of thesetreatments. Follow-up duration was 30 months in the patients treated with nusinersen and 18 months in patients treated with risdiplam. Renal function was determined by analyzing serum creatinine, cystatin-C and potassium levels and urine samples for protein and B2-microglobulin levels. In a subset of patients, we analysed 24-hour urine collections for protein levels and sodium, potassium, phosphate, uric acid, oxalic acid, citrate and calcium concentrations.

We enrolled 266 patients with SMA types 1-4. Median age was 32 years (range 22.2-49.1), creatinin levels were too low to reliably determine glomerular filtration rates (eGFR). Using serum cystatin C as an alternative, 54 (21%) patients had eGFR rates <90ml/min/1.73m2, indicating an increased risk of developing chronic kidney failure. Based on serum cystatin C, ten (4%) patients had eGFR compatible with chronic kidney failure (eGFR <60ml/min/1.73m2) and two patients had end-stage renal failure. 144 (51.1%) patients had abnormal serum potassium levels (<3.8 mmol/L). Urine of 55 patients (22%) contained increased protein levels. 44 (16.5%) patients had a history of kidney stones or nephrocalcinosis. Urine collection samples did not show abnormal levels of calcium, citrate, oxalic acid or uric acid and serum calcium, and urine pH levels were within the normal range. Treatment with *SMN2* splicing modifiers (i.e. nusinersen or risdiplam) resulted in significant reduction of the number of patients with hypokalemia (55.1% to 26.1% in nusinersen group and, 56% to 42.4% in risdiplam group), but no reduction in proteinuria. Cystatin C eGFR decreased during follow-up in patients treated with both nusinersen and risdiplam.

Renal function is vulnerable to low levels of SMN. Patients with SMA are at risk of decreased renal function, which may go unnoticed if analyzed using serum creatine. Renal clearance should therefore be monitored using cystatin C rather than creatinine. Serum and urine analysis of SMA patients indicate abnormal tubular function. SMN augmenting therapies may improve tubular dysfunction, but not renal clearance capacity.

P57

Natural history of pulmonary function in adult patients with Spinal Muscular Atrophy type 2 & 3

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The availability of disease-modifying treatment for adult patients with Spinal muscular atrophy (SMA) has emphasized the need for natural history data, including those on respiratory function.
Aims of the study were to describe the annual progression of pulmonary function in untreated adult patients with SMA types 2 and 3 and to investigate its correlation to patients' functional motor status.

This was a retrospective observational natural history study of untreated adult patients regularly followed by the five Italian centres and one US centre included in the International SMA Consortium (iSMAc).
Anthropometric data, absolute and percent predicted forced vital capacity (FVC, FVC% pred.), peak cough flow (PCF, l/min), non-invasive ventilation (NIV) and cough-assistance requirements were collected.
Cross-sectional and longitudinal analyses of respiratory function across SMA types and motor functional groups were conducted.

One hundred ninety patients were included (SMA type 2, n = 80, median age 25.9 years, IQR: 19.2-35.7; SMA type 3, n = 110, median age: 35.1 years, IQR: 24.3-45.9). The median follow-up duration was 3.2 years.
At first assessment, absolute and percent predicted FVC and PCF were significantly different ($p<0.0001$) in SMA type 2 compared to type 3, and across motor functional status groups within each SMA type.
FVC% progressed annually by 0.09% in SMA type 2 and by 0.53% in type 3. This progression was not significant over time and did not differ according to motor functional groups.
Seven of the 35 patients (20%) with an FVC% pred. above 60% dropped below this threshold at a median age of 61 years.

These results broaden the characterisation of the respiratory involvement in untreated adults with milder forms of SMA. The novel information provided by this study suggest that the progression of pulmonary function in this cohort is relatively slow even over long-term observation and is disjointed from motor function. This should be taken into account when assessing the impact of disease-modifying treatments on pulmonary function.

P58

Impaired pattern of S-glutathionylation and redox enzymes in spinal muscular atrophy mouse model

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Spinal muscular atrophy (SMA) is a neuromuscular disease, characterized by loss of lower alpha motoneurons, which leads to proximal muscle weakness. SMA is caused by reduced levels of Survival of Motor Neuron (SMN) protein due to biallelic deletions or mutations in the *SMN1* gene and primarily non-functional *SMN2* gene copies, which each patient carries in the genome. When SMN levels fall under a certain threshold a plethora of cellular pathways are disturbed and thus, SMA has been characterized as a multi-organ disorder. While three treatments for SMA have been approved, that aim in restoration of SMN levels in CNS and systematically, there is a need for combinatorial therapies including SMN-independent strategies. Amongst the molecular pathways that are disturbed in SMA, the most prominent include impaired mitochondria homeostasis, oxidative stress, systemic hypoxia, decreased mRNA translation and impaired actin cytoskeleton. Intriguingly, core molecules of these pathways belong to S-glutathionylation target proteins (PSSG). We initiated our investigation using the Taiwanese mouse model of SMA and by performing a multi-organ analysis of the S-glutathionylated proteome, which revealed an overall reduction of PSSG levels in SMA compared to control group organs. Additionally we showed that core redox enzymes that regulate S-glutathionylation are down-regulated in brain, spinal cord, liver, heart and several muscles of SMA mice. We also showed that upon injection of the SMA animals with SMN-antisense oligonucleotides the levels of S-glutathionylation are returning back to normal levels as well as some of the enzymes that regulated this mechanism. We aim, to elucidate whether this de-regulation of the redox system stems from glutathione metabolism deregulation and which SMN-dependent or independent mechanisms manifest this alteration by utilizing proteomics and biochemical assays in multiple organs of the SMA mouse model.

P59

Investigating the role of SMN1 in R-loop formation during Spinal Muscular Atrophy (SMA) pathogenesis

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R-loops are RNA:DNA hybrids with many cellular functions. In homeostasis, they are thought to play a role in gene and chromatin regulation. However, pathological R-loop accumulation is a feature of several diseases, including motor neuron disorders such as Spinal Muscular Atrophy (SMA). In *vitro*, acute (knockdown) and chronic (SMA) SMN-deficiencies cause the accumulation of co-transcriptional R-loops and DNA damage leading to genomic instability and neurodegeneration. However, how and when the building up of R-loops occurs during SMA remains poorly understood, as well as the precise molecular role of SMN1 in this process. With these questions in mind, we sought to better understand the formation of R-loops in a severe SMA mouse model (SMNdelta7) and patient-derived SMA myoblasts.

R-loop accumulation was assessed through immunofluorescence (IF) using the S9.6 antibody in tissues from the SMNd7 mouse model and patient-derived myoblasts. To create a model of acute *SMN1* loss, we utilized a dox-inducible shRNA. Gene expression was determined using RNA sequencing.

We characterized the accumulation of R-loops throughout disease progression in the SMNd7 mice. We observed a 2-fold increase in R-loops at postnatal day 3 (P3), before motor symptom onset, which was maintained at the disease end-stage, P14. We also investigated the level of R-loops in myoblasts derived from SMA Type I and Type II patients, and confirmed their accumulation only in Type I cells, suggesting an SMN1 protein dose-dependent mechanism. We are now establishing their progression in the central nervous system *both in vivo* and *in vitro*. To confirm the direct link between SMN1 loss and R-loop accumulation, we used an inducible shRNA knockdown strategy to acutely deplete *SMN1* in muscular and neuronal cell lines. A 2-fold increase in R-loops was observed by immunostaining after 48 hours of depletion, followed by the restoration of the basal R-loop quantity after 24 hours without doxycycline. Next, we sought to understand the molecular mechanism by which loss of SMN1 causes R-Loop accumulation. We hypothesized that SMN1 could affect the splicing of various R-loop interacting factors. Of the 30 differentially expressed genes identified in the SMNd7 mouse *tibialis anterior* muscle, we are focusing on the ones involved in replication stress prevention and/or resolution. Ongoing work seeks to understand the role of these genes in R-loop accumulation.

Collectively, these results establish that R-Loop accumulation occurs in mouse SMA muscle prior to the onset of motor symptoms. We confirmed these findings in Type I SMA myoblasts, but saw a dose-dependent effect of SMN1, as Type II patients did not have R-loop accumulation. Ongoing work is determining the molecular mechanism by which SMN1 loss causes this accumulation.

P60

Posttranslational regulation of the Survival Motor Neuron (SMN) complex

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Splicing is a crucial event in gene expression during which introns are removed, leaving only coding exons. The Survival Motor Neuron (SMN) complex plays a pivotal role in assembling spliceosomal small nuclear ribonucleoproteins (snRNPs). Depletion of SMN protein is known to disrupt these pathways, impacting human well-being. While numerous posttranslational modifications (PTMs) within snRNP constituent proteins have been described, a lack of suitable methods for site-specific installation has limited our understanding of their functions

In this study, we focus on investigating the role of PTMs and their effects on the SMN complex. Recently, we synthesized four peptide segments of the SMN protein with different PTMs using solid-phase peptide synthesis (SPPS) and connected them via native chemical ligation (NCL). Total chemical synthesis through SPPS offers full flexibility for modifying SMN variants, which will then be incorporated into the SMN complex for in vitro studies. These studies aim to analyze the impact of these modifications on SMN's function in UsnRNP assembly.
Additionally, we seek to understand more about the factors required for stable complex formation during the splicing process. For this, we recently expressed the N-terminal portion of SmD1 protein, and we are currently using expressed protein ligation (EPL) to ligate different C-terminal peptides carrying variable numbers of symmetrically dimethylated arginines (sDMA). By unraveling the intricate web of PTMs within the SMN complex and their impact on UsnRNP assembly, our research not only contributes to a deeper understanding of gene expression processes but also offers a potential avenue for therapeutic intervention in SMA. These insights may pave the way for novel treatment strategies aimed at modulating PTMs to ameliorate the devastating effects of SMA, bringing hope to affected individuals and their families.

The elucidation of PTM-driven mechanisms associated with the SMN complex holds great promise for understanding the pathogenesis of Spinal Muscular Atrophy (SMA). SMA is a debilitating neuromuscular disorder characterized by the degeneration of motor neurons, and emerging evidence suggests that PTMs may play a critical role in SMA progression. By unraveling the intricate web of PTMs within the SMN complex and their impact on UsnRNP assembly, our research not only contributes to a deeper understanding of gene expression processes but also offers a potential avenue for therapeutic intervention in SMA. These insights may pave the way for novel treatment strategies aimed at modulating PTMs to ameliorate the devastating effects of SMA, bringing hope to affected individuals and their families.

P61

Decoding the role of oxidative stress in spinal muscular atrophy

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Spinal Muscular Atrophy (SMA) is the most prevalent paediatric cause of lower motor neuron disease and the primary monogenic factor contributing to infant mortality. Although it is broadly known that depletion of the ubiquitously expressed Survival Motor Neuron protein (SMN) is the cause of this disorder, there are still unresolved questions regarding the signalling pathways involved in SMA and their relationship to disease pathology. Oxidative stress (OS) in SMA has been noted in various SMA models that describe mitochondrial defects and increased reactive oxygen species (ROS) production. However, a comprehensive overview of how oxidative stress is perturbed in SMA is missing. The aim of this project is to delineate whether oxidative stress contributes to SMA pathogenesis.

Here, we utilize the powerful genetic tools of *Caenorhabditis elegans* in a previously established *C. elegans* SMA model by employing a range of behavioural and pharmacological assays to delineate the mechanism(s) that connect oxidative stress perturbations and SMN function.

Exposing the *C. elegans* SMA model to compounds that induce oxidative stress by increasing intracellular superoxide levels significantly reduced the survival of the animals, indicating an increased sensitivity to oxidative stress. Currently, we are using genetic, pharmacological, and behavioural assays to study the impact of ROS levels in the *C. elegans* SMA neuromuscular phenotypes.

The goal of this study is to identify the cellular and molecular pathways needed to spearhead further therapeutic avenues for SMA treatment options. Altogether, we emphasize the strengths of *C. elegans* as an exceptional tool to comprehend the molecular mechanisms underlying this devastating motor neuron disease.

P62

Adipokines and spinal muscular atrophy: Results from a Multicentric study in Italy

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Patients with spinal muscular atrophy (SMA) are characterized by impairment of nutritional status due to the magnitude of neuromuscular disorder. SMA children, particularly SMA1 and SMA2 low functioning, waste due to a significant increase in Body Fat (BF) and a significant reduction of fat free mass disproportion. Leptin and adiponectin are the most abundant adipose tissue biomarkers related to the body fat amount and to glucose and lipid metabolism. The aim of this study was to describe serum leptin and adiponectin concentrations in a sample of SMA 1 and 2 to evaluate their association with anthropometric parameters and body compositionin.

To investigate the associations of leptin and adiponectin with body composition and glucose and lipid metabolism impairment we recruited naïve 39 SMA children (7 SMA1 and 32 SMA2,). Mean ages were 4.7 (0.8–13.0) and 4.9 (1.7–11.6) years, respectively. All patients underwent a clinical and a comprehensive nutritional assessment including anthropometric measurements, Dual-energy X-ray absorptiometry (DXA), fasting blood sample (including serum glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides (TG), insulin, leptin and adiponectin). The homeostatic model assessment-insulin resistance (HOMA-IR) and atherogenic indexes (TC/HDL, LDL/HDL, TG/HDL) were calculated.

53% and 56% of children were underweight according to WHO percentiles, respectively; while only 6% using SMA-specific percentiles. The percentage of obese children was ≈6%. No SMA patient showed a percentage of fat mass by DXA lower than reference values. 44% of patients had alterations in at least one of the 5 parameters selected for dyslipidemia. Mean serum fasting glucose and HOMA-IR were 83±10 mg/dl and 0.95±0.70, respectively. None of the children showed fasting glucose >100 mg/dl, while 8% of them showed fasting glucose <70 mg/dl. 3% of the sample showed HOMA-IR >2.5.

Leptin (30026±52697 pg/ml) showed a positive correlation with bmi-z-WHO ($\rho=0.382$; $p<0.05$), bmi-z-SMA ($\rho=0.600$; $p<0.05$), and fat mass index ($\rho=0.676$; $p<0.001$), with an exponential trend above 40% of fat mass. Moreover, a negative correlation with LDL-HDL ratio ($\rho=-0.353$; $p<0.05$) was found. There were no other significant associations found, not even with adiponectin.

We found a higher prevalence of lipid abnormalities but no impairment of the glucose profile. Leptin shows promise as a potential biomarker for body composition and dyslipidemia in SMA. By contrast, adiponectin was not correlated with anthropometric variables and body fat.

P63

Analysis of glycinergic system alterations in Spinal Muscular Atrophy

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Spinal Muscular Atrophy (SMA) is a neurodegenerative disease due to *SMN1* gene mutation causing lower motor neuron (MN) loss and several peripheral alterations. Although gene-based treatments have improved SMA course, the available therapies still show important limitations. Therefore, the identification of novel therapeutic strategies is needed.
Recently it has been observed that SMA mitochondria show remarkable morphological and functional alterations (similar to those described in Parkinson's Disease, Alzheimer's Disease and Amyotrophic Lateral Sclerosis) and therefore these organelles can be considered promising targets for SMA therapy.

An anticorrelation-based bioinformatic method was exploited to detect genes implicated in mitochondrial functions potentially relevant for drug repositioning approaches. Molecular analysis (RT-PCR and Western Blot) were performed both in vitro (on *Smn*-silenced NSC34, a MN cell line) and in vivo (on SMNdelta7 mice, representative of a severe model of SMA), to evaluate gene and protein expression. Finally, RNAScope for in situ hybridization (ISH) and immunofluorescence (IF) reactions revealed the specific location of the gene/protein of interest; morphological analyses were also performed by Neurolucida software on lumbar spinal cord slices, to detect early and late alterations in spinal interneurons.

Through bioinformatic analysis we identified 8 mitochondrial genes significantly *SMN1*-anticorrelated, in physiological conditions. To investigate which of them could be related to SMA, we performed RT-PCR on SMNdelta7 mice tissues. We found that *Gcsh*, a gene codifying for a glycinergic cleavage system (GCS) subunit located in mitochondria, is significantly upregulated in lumbar spinal cord of SMA pups, since an early symptomatic stage (postnatal day 5, P5). Western Blot analysis confirmed the trend of the related protein upregulation. Furthermore, RNA-Scope ISH showed a significant *Gcsh* increase in SMA MNs and the same trend was observed in *Smn*-siRNA silenced NSC34 cells. Consistently, IF staining on spinal cord slices confirmed a significant higher expression of GCSH in MN soma. Finally, to analyze further glycinergic component alterations, we focused on morphological analysis of Renshaw Cells, glycinergic interneurons involved in MN recurrent inhibition. As a result, we observed a cellular shrinkage since P5 that worsens in the late stage of the disease (P12).

These findings raised the possibility that the glycinergic system could be affected in SMA. In particular, we hypothesized that a reduction in MN inhibition due to a possible higher glycine degradation could contribute to their hyperexcitability and their following loss.

Further analysis on GCS role (in particular in MNs) and on glycinergic pathway could be instrumental for a better understanding of the mitochondrial role in SMA and to identify new molecules for SMA complementary therapy.

P64

Unravelling the role of GABA signalling and metabolism (dys)regulation in Spinal Muscular Atrophy: Results from SMAΔ7 mice cortex

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Spinal muscular atrophy (SMA) is a genetic neuromuscular disease affecting children and young adults, characterized by motor neuron (MN) impairment and skeletal muscle atrophy. However, we also observed a selective degeneration of motor cortex (CRTX) layer V pyramidal neurons in SMAΔ7 mice (a severe SMA murine model), compared to WT controls, suggesting that its pathogenesis is likely more complex than previously anticipated. To date, although the monogenic causes of the disease are well known, many other aspects are still unclear, and the available therapies still show many limits. Intriguingly, neuroprotective effects of GABA-targeting drugs were reported in SMA, suggesting a possible dysregulation of GABA (the main inhibitory neurotransmitter of the central nervous system) and inhibitory interneuron (IN) pathways at cortical level, as a common aetiology shared with other neuronal diseases.

We have strong preliminary results showing perturbation of GABA metabolism and Parvalbumin (PV) IN functions in SMAΔ7 mice sensorimotor (SM) CRTX, in the late disease stage (postnatal day 12), in comparison with WT. By immunofluorescence (IF) analysis, we observed a significant reduction of GABAergic signal (-57%, $p<0.01$) and reduced density of GABA+-cells (-25%, $p<0.01$) in SMAΔ7 SM CRTX, along with an impaired distribution and reduction of GAD65 and GAD67 (GABA synthesis enzymes) signal (-60% and -65%, respectively, $p<0.05$) and GAD67+-cells (-20%, $p<0.05$), underlying neurotransmitter synthesis defects. Moreover, PV INs were found significantly reduced in number (-34%, $p<0.05$) and morphologically altered, suggesting possible failure in their inhibitory functions. Immunoblotting further confirmed a reduction in GAD65/67 protein levels in the SMAΔ7 SM CRTX (-25%, $p<0.05$). Furthermore, IF analyses in SMAΔ7 motor CRTX showed a reduction of GABAergic synapses contacting-neurons in layers II/III (-28%, $p<0.01$) and V (-38%, $p<0.001$), suggesting loss of GABAergic contribution to cortical excitatory/inhibitory homeostasis.

Overall, these results show for the first time GABAergic dysregulations in the SM CRTX of SMA mice, possibly contributing to the onset of the disease. Further studies aimed at fully understanding and pharmacologically rescuing GABA pathways will pave the way for new SMA treatments.

P65

Multi-dimensional genome-wide analysis reveals robust pre-symptomatic and model-independent defects in translation in three sma mouse models

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Dysregulation of protein synthesis contributes to a range of neurodegenerative conditions, including Spinal Muscular Atrophy (SMA). Recent research has revealed that in the Taiwanese mouse model of SMA, SMN deficiency leads to defective protein synthesis in primary motor and cortical neurons, and in numerous tissues at early and late stages of disease. SMN binds to ribosomes and regulates the translation of a subset of transcripts relevant to the disease pathogenesis, which showed defects in ribosome occupancy upon SMN loss. Whether these defects are present in different mouse models and at different stages of disease progression in multiple tissues is still unclear.

To understand whether SMN loss might be related to model-, tissue- and stage-specific translational impairments, we used polysome profiling and measured the fraction of ribosomes in polysomes (FRP) in the brain, spinal cord, and liver at pre-, early, and late stages of SMA in the Taiwanese, and in the milder Δ7, and Smn2B/- mouse models. We performed co-sedimentation profiles to inspect the association of the SMN protein with the ribosomal compartments in control and SMA. To identify genes displaying altered ribosome occupancy and detect common gene regulatory patterns, we coupled ribosome profiling data obtained from various models, tissues and stages with gene network expansion analysis.

We observed a decreasing trend in the fraction of ribosomes in polysomes in all mouse models, confirming that translation impairments also occur in mild SMA models. In all models, tissues and stages this result was further reinforced finding that SMN does not associate with the translational machinery in SMA condition. Intriguingly, differential analysis of ribosome profiling showed remarkable translational changes in the Taiwanese mouse brain at the pre-symptomatic stage compared to the early-symptomatic stage. In milder mouse models, we observed significant translational abnormalities in brain, spinal cord, and liver at the pre-symptomatic stage, further supporting the hypothesis that translational defects are model-independent. Poor overlaps of genes displaying altered ribosome occupancy have been found between different stages, tissues, and models. Given this specificity, we wondered whether instead of common genes, these translational defects are describing the common network-based interactions between genes that underlie shared mechanisms relevant for disease pathogenesis. Using gene network expansions based on genes with altered ribosome occupancy, we identified populations of interacting genes that are disrupted in SMA pathogenesis in a stage-, tissue-, and model-independent way.

Collectively, our results demonstrate that robust pre-symptomatic and model independent translation alterations occur in SMA across models, tissues and stages, and supports the idea that gene networks can be used to identify therapeutic targets.

P66

A novel bootstrap approach for gene set enrichment analysis with transcriptomics and proteomics data from studies on Spinal Muscular Atrophy

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Pathway databases such as PANTHER or REACTOME as well as Gene Ontology (GO) annotations are curated either manually or by an algorithm. As a result, gene set composition remains uncertain. The aim of this project is to develop improved methods for gene-set enrichment analysis (GSEA) and to apply them to multi-omics data from Spinal Muscular Atrophy (SMA) research. Omics analyses have become increasingly important to unravel several molecular mechanisms underlying various diseases, such as SMA. SMA is a complex disease affecting multiple organs and involving various molecular mechanisms. Robust pathway analysis that considers the uncertainty in gene-set annotations can help ensure that the results of omics analyses are reliable and reproducible.

Kidney tissue of pre-symptomatic postnatal day 3 (P3) of severe (”Taiwanese”) SMA mice was used for transcriptomic and proteomic analyses. Differential expression analysis was performed on the data. We resampled the genes iteratively from differential expression analysis using the bootstrapping (subset of genes) approach for each gene set or pathway to study the variability of results. Furthermore, we aggregated the results from each bootstrap run by merging the ranking lists of gene-sets by rank aggregation approach. A score provided by rank aggregation can then rank the gene-sets newly. In addition, we combined these results from different omics levels to analyze the robustness on a multi-omics level. An R-package ‘bootGSEA’ that implements the proposed method and provides graphical views of the findings has been implemented.

We demonstrate the usability of our novel approach for GSEA on transcriptomics and proteomics data from a study on SMA in kidney of a severe (“Taiwanese”) SMA mouse model. SMN is a part of the SMN complex and functionally interferes with several other complexes, such as the cytoplasmic ubiquitin ligase complex (GO:0000153), which is highly ranked when ordered by their integrated score in our GO cellular component analysis. Therefore, our new pipeline provides not only robust terms, but also biologically relevant terms when ordered by their integrated scores.

Using our R-package bootGSEA, we were able to identify gene or protein sets that were less robust when the set composition changed during the bootstrap analysis. The top hits at the multi-omics level included complex terms that are relevant in SMA disease mechanisms. GO terms with a larger rank difference between the original and bootstrap analyses should be excluded to avoid misinterpretation of the results. Overall, the robustness analysis highlights the importance of using appropriate methods for GSEA and taking into account the potential variability in gene or protein set annotations in different databases, which can increase the reproducibility and reliability of results.

P67

Dysfunction of $K_{ir4.1}$ in spinal astrocytes leads to MEK/ERK-mediated BDNF-induced overexpression of GluA1 AMPA-receptors in motor neurons during the pathogenesis of late-onset spinal muscular atrophy

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Spinal Muscular Atrophy (SMA) is a genetic disorder characterized by progressive muscle weakness. It is caused by a deletion of the *SMN1* (survival of motor neuron) gene located on Chromosome 5. Insufficient SMN protein leads to the loss of spinal motor neurons. Emerging evidence suggests the involvement of non-neuronal cells, such as astrocytes, in the pathogenesis of SMA.
Astrocytes play a crucial role in maintaining the extracellular milieu, and damage to them can result in functional alterations of spinal motor neurons.
Using a translational approach, this study aimed to investigate the interaction between spinal astrocytes and motor neurons, focusing on the inward rectifying potassium channel $K_{ir4.1}$ and the neurotrophin BDNF (brain-derived neurotrophic factor).

Using a mouse model of late-onset SMA, cell cultures, and tissue cultures, the expression and function of $K_{ir4.1}$ and its influence on BDNF expression/release were examined using electrophysiology, immunohistochemistry, western blot and PCR analysis. Analysis of potential signaling pathways was performed using specific inhibitors. In a translational approach, human induced astrocytes, and liquor samples of SMA patients were used.

A reduction in $K_{ir4.1}$ expression and function in spinal astrocytes was observed. This led to calcium-mediated activation of the MEK/ERK signaling pathway, resulting in increased expression/release of BDNF. Spinal motor neurons exhibited elevated expression of BDNF receptors (Trk-B) at an early stage. Additionally, BDNF led to enhanced expression of GluA1 (Glutamate AMPA receptors) in spinal motor neurons.

The observed early activation of spinal astrocytes and the reduction of $K_{ir4.1}$ protein as well as functional changes suggest a significant role in the pathogenesis of SMA. We identified five different targets that directly demonstrated activation of astrocyte-associated MEK/ERK/BDNF-signaling pathway and could potentially serve as an SMN-independent therapeutic target. Substances targeting key proteins in this pathway are for example already utilized for therapeutic purposes in other diseases (e.g., tumor diseases).

P68

Alterations along the neuroendocrine axis of leptin homeostasis: fat tissue and hypothalamus in a severe SMA mouse model

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Spinal Muscular Atrophy (SMA) was long solely considered as a neurodegenerative disease with a predominant neuromuscular phenotype. Recently, SMA has been described as multi-system disorder with new multi-organ phenotypes. Although therapies have shown to be effective, there is yet no cure for SMA. Consequently, the need for a complementing therapy addressing peripheral signatures of novel SMA phenotypes increases. Previous studies of infant SMA patients revealed increased leptin levels in blood and urine, indicating disturbed fat and energy homeostasis. Leptin regulates energy metabolism and fat homeostasis and is expressed in fat tissue as a signal of saturation by binding to receptors in the hypothalamus. In development, leptin acts neuroprotective supporting neuronal and glial cell proliferation and maintenance. In this study, we hypothesize that leptin is dysregulated in SMA and tissues show molecular signatures of altered energy metabolism and fat homeostasis.

Murine samples of hypothalamus and white fat tissue were dissected from the severe Taiwanese SMA mouse model at pre-symptomatic (P1), early-onset (P3, P5) and highly symptomatic (P7) postnatal stages. RNAseq and proteomics data of hypothalamus and fat tissue were analyzed by Ingenuity Pathway Analysis (IPA). Additionally, leptin transcript and protein level was quantified by qRT-PCR and Western blot.

At early-onset (P3) of SMA, leptin protein expression was negatively correlated with the body weight of SMA mice compared to healthy littermates. Additionally, analysis of both transcript and protein levels of leptin at P3 in SMA mice suggested general changes in fat metabolism. Proteomics and RNAseq confirmed molecular alterations and altered signaling pathways of the energy metabolism and fat homeostasis at pre-symptomatic stage P1 in SMA mice. In addition, targets regulating actin dynamics and cell migration have been found to be dysregulated.

Taken together, our results indicate disturbances within the energy metabolism and fat homeostasis on the molecular level at pre-symptomatic and early stages in SMA, suggesting changes during the development before SMA symptoms evolve. Thus, these findings indicate the need for complementary treatment of novel SMA phenotypes in the periphery targeting the energy metabolism.

Newborn Screening

P69

Six months of newborn screening for spinal muscular atrophy in Croatia

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Spinal muscular atrophy (SMA) is a neuromuscular and neurodegenerative disease caused by homozygous deletion of exon 7 of the *SMN1* gene in 95 % of cases. Until recently, SMA was the most common genetic cause of death at an early age. However, with the development of therapies that modify the natural course of the disease, the prognosis of patients with SMA has significantly improved. All three existing therapies, nusinersen, onasemnogene abeparvovec and risdiplam, are available in the Republic of Croatia and applied according to the indications of the Croatian Health Insurance Fund. The best outcomes in treatment are achieved if the therapy is applied before the symptoms of the disease appear, which is why newborn screening (NBS) for SMA is of key importance in the treatment process. It is expected that a certain number of patients detected by NBS will already be symptomatic at the time of diagnosis. A small number of patients, around 5 %, cannot be detected by NBS.

Herein, we present the results of the SMA NBS during the first 6 months of the pilot project in Croatia and verify the suitability of the Targeted qPCR SMA assay (ZenTech, Belgium) for SMA NBS.

The first-tier test for SMA NBS developed by ZenTech is based on quantitative polymerase chain reaction (qPCR) from a dried blood spot and detects homozygous deletion of exon 7 of the *SMN1* gene. The second-tier test is based on MLPA technique (MRC Holland, P021 kit).

The pilot project of SMA NBS in Croatia started on March 1st, 2023 in the Dept. of Laboratory Diagnostics of University Hospital Centre Zagreb. By September 1st, 2023, a total of 16 035 newborns were tested. 0.05 % of parents / guardians refused screeening. Two SMA patients were detected and their diagnosis was confirmed by MLPA analysis on the 11th and 12th day of life, respectively. One patient had 3 *SMN2* copies, and the other had 6 *SMN2* copies. There have been no false positives or false negatives to our knowledge so far. The incidence of SMA of 1 in 8 017 determined during the NBS pilot study in Croatia is consistent with previous studies of SMA prevalence in Croatia. SMA NBS allows the early diagnosis of SMA as well as timely application of therapy, which prevents disease progression. Our results indicate that the ZenTech assay can be reliably used in SMA NBS, as well as the importance of adding SMA in the national screening program of the Republic of Croatia.

P70

Revolutionizing Spinal Muscular Atrophy Prevention in Serbia: Implementing a Mandatory Statewide Newborn Screening

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Spinal muscular atrophy (SMA) is the prevalent genetic cause of childhood mortality. Pioneering treatments yield utmost advantages only within the presymptomatic phase, underlining the medical and ethical significance of newborn screening.

In 2022, the Centre for Human Molecular Genetics initiated a pilot study of the newborn screening for SMA, working closely alongside the University Children's Hospital Tirsova and Association SMA Serbia. The aim was to lay the foundation for the implementation of statewide newborn screening for SMA in Serbia by conducting screening for ~8000 infants from the Obstetrics and Gynaecology Clinic Narodni Front over the course of a year. In the subsequent year, we expanded the initiative to include another maternity hospital located outside Belgrade, introducing sample shipment via postal services and extending screening accessibility to a greater number of infants.

In the initial year, 6 950 newborns underwent testing, revealing SMA in two unrelated infants. Subsequently, an older sibling of the first newborn, although asymptomatic at the time, was also tested at the age of 16 months, and SMA diagnosis was confirmed in this child as well. All three children received therapeutic interventions in <1 month from birth. To date, they have exhibited no signs of SMA, and there have been no false-negative outcomes among the newborns who tested negative during the screening. In the second year, an additional 5 000 newborns underwent testing.
As frontrunners in this field in Serbia, we orchestrated harmonized efforts across various tiers of healthcare, established screening and diagnostic algorithms and follow-up protocols. Our extensive efforts were primarily aimed at elevating awareness among all stakeholders about the critical importance of early disease detection. In this transformative journey, we transitioned from being isolated individuals and visionaries who championed a singular idea to an entire community and nation that now acknowledges the paramount significance of newborn screening. As a result, a total of 11 950 infants underwent testing during the 17-month pilot project, culminating in the rapid incorporation of newborn screening for SMA into the national screening program, effective as of September 14th 2023.

Timely detection and treatment can transform SMA into a manageable condition, and there is substantial evidence supporting its inclusion in state-wide screening programs.

P71

Five years follow up of children identified by newborn screening in Southern Belgium

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The three drugs approved to treat spinal muscular atrophy (SMA) have all shown much better efficacy when they are administered early- in ideally pre-symptomatic patients. This has inspired several newborn screening pilot projects. PCR-based universal newborn screening program for SMA was established in Belgium on March 5th, 2018. By October 1, 2023, of the 281,000 newborns screened, 20 patients were identified with SMA and treated early with a median age at 35 days (between 18 and 54 days). We consider here the real-life functioning and clinical evolution of all children screened and treated since the start of the program.

Ethical approval (number B412201734396) was obtained from the Institutional Review Board (Ethical Committee, CHR Citadelle Hospital, Liège, Belgium) in accordance with the Declaration of Helsinki. All patients were examined by a board-certified pediatric neurologist with experience in SMA. At the first visit, parents were informed of various treatment options. Phenotype at the start of treatment and age at sitting and walking were recorded. Longitudinal motor milestone assessment was performed by trained physical therapists, CHOP-INTEND and HINE-2 scales were performed during the first two years of age.

The median time of follow up is 29 months after birth (between 2 and 64 months). Nine patients have 2 copies of *SMN2*, five have 3 copies and six of them have 4 copies. Four of the nine patients with 2 copies of *SMN2* were deemed symptomatic at the first consultation. Six patients were treated with nusinersen- two shifted to risdiplam and 1 shifted to onasemnogene abeparvovec, seven with risdiplam and seven with onasemnogene abeparvovec. The median turn-around time for diagnosis was 17 days after birth and the median delay for first visit was 20 days of life.

We will present the age of motor acquisition: sitting, standing up and walking with and without assistance for each patient.

P72

5qSMA newborn screening in Portugal: 10-month experience

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Newborn screening (NBS) has been implemented in many countries for various metabolic and endocrine diseases. Since the advent of disease modifying treatments (DMT), 5q Spinal Muscle Atrophy (5qSMA) fully meets the modified criteria proposed by Wilson and Jungner, that determine whether screening for a disease should be included in an NBS panel. Approximately 95-98% of patients present with homozygous absence of exon 7 of *SMN1*, which is identified in 5qSMA NBS assays. In recent years 5qSMA NBS programs have been implemented worldwide. In Portugal, a pilot NBS program has been implemented on the 27th of October 2022.

Retrospective multicenter longitudinal study. Epidemiologic and clinical data from all 5qSMA identified patients were analyzed.

From October 27th 2022 until the 31st of August 2023, 72426 newborns born in Portugal were screened. Five 5qSMA patients were identified (3 boys, 2 girls). To date there are no other new diagnosed 5qSMA patients who underwent NBS in Portugal. Samples were received between the 5th and 10th day of life and initial screening result was obtained between day 8 and day 14. All specimens were retested using Multiplex Ligation-dependent Probe Amplification (MLPA) technique and exon 7 *SMN1* homozygous deletion was confirmed. In these cases, *SMN2* copy number was determined. Confirmation diagnosis was obtained between the 11th and 20th days of age. Two patients had 2 *SMN2* copies, two patients had 3 *SMN2* copies and one patient had 5 *SMN2* copies. Four patients were asymptomatic in the first visit, although one of them later became symptomatic. Four patients were treated, two with onasemnogene abeparvovec and two with nusinersen (one in trial). Treatment was initiated between the 14th and the 34th day of age (two symptomatic, two asymptomatic). One patient had anti-AAV 9 antibodies. Baseline CHOP-INTEND of *SMN2* 2 copies patients were 49/64 and 16/64. CHOP-INTEND increased during follow-up period up to 54 and 30 respectively, however there is still a significant delayed milestone achievement. Both patients with 3 *SMN2* copies were treated and are still asymptomatic (8-month and 6-month follow-up period).

All SMA DMT trials revealed that early treatment seems to be crucial for maximizing therapeutic effects, ideally pre-symptomatically. Although it's a very small cohort our experience emphasizes that detection of disease in NBS does not allow presymptomatic treatment in all patients. An effort in reducing the time between the final result of the screening test and the onset of the therapy should be made. No false negatives or positives results were obtained to date reinforcing the high specificity and sensitivity of the test.

P73

Effect of newborn screening on infants with SMA – a population-based, non-randomized, parallel group study within the SMArtCARE registry

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Early diagnosis and treatment are key for the clinical outcome in infants with spinal muscular atrophy (SMA). Therefore, newborn screening (NBS) programs have been implemented to detect the disease before onset of symptoms in several countries. In Germany, a regional pilot project was completed prior to the implementation of the nationwide NBS. This allowed a direct comparison of data obtained from children diagnosed through NBS with those diagnosed after symptom onset from the same population and birth cohort.

We performed a population-based, non-randomized, parallel group study within the SMArtCARE registry. All children born between January 2018 and September 2021 with a genetically confirmed SMA and up to three *SMN2* copies were included.

We identified 234 children who fulfilled the inclusion criteria: 44 children diagnosed through NBS (18.8%) and 190 children diagnosed clinically after onset of symptoms (81.2%). Mean age at start of treatment was 1.26 ± 2.2 months in the NBS cohort and 10.72 ± 9.05 in the clinically diagnosed cohort. In the NBS cohort, 90.9% of children gained the ability to sit independently vs. 74.2% in the clinically diagnosed cohort (Log-rank $p<0.0001$). For independent ambulation, the ratio was 63.6% vs. 14.7% ($p<0.0001$), respectively. The probabilities of the need for ventilator support or tube feeding and adverse events were significantly lower in the NBS cohort.

This is the first population-based, controlled study to confirm the benefit of NBS for SMA in a real-world setting. Functional outcomes and thus the response to treatment were significantly better in the NBS cohort compared to the unscreened control group.

P74

Effectiveness of the "patient/doctor driven" SMA registry based on the analysis of neonatal screening results in Ukraine

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The Ukrainian SMA Registry is a disease-specific and geographically restricted instrument for spinal muscular atrophy (SMA) epidemiologic studies. The Registry aims to register all cases in the population and to collect uniform data to evaluate different types of outcomes, is based on the TREAT NMD Core Data set for SMA and serves scientific, clinical, and health policy purposes. The Registry is "patient/doctor-driven", i.e., it collects data directly from both the patient and the doctor. In October 2022, expanded neonatal screening (NBS) for 21 rare diseases, including SMA, began in Ukraine. An analysis of the Registry using 11 months of screening data could be useful for predicting the efficacy and completeness of data.

Since the launch of the NBS, 127400 examinations have been carried out by 4 neonatal screening centers as of September 2023, including: 54100 by the Lviv center, 46800 by the Kyiv center, 11700 by the Kharkiv center, and 14800 by the Kryvyi Rih center. Among these, SMA cases were found in newborns as follows: Lviv (8), Kyiv (4), Kharkiv (2), and Kryvyi Rih (3). The largest group of patients (7) had 3 copies of the SMN2 gene; 5 patients had 2, 3 patients had 4, and one patient each had 1 and 5. Between November 2022 and September 2023, the NBS detected 17 patients over11 months. Of these, 4 (those with 4+ copies of SMN2, out of 17) are asymptomatic, accounting for 23.53%. In 2023, the registry included 8 patients detected through the NBS, including 4 asymptomatic patients, and 4 registered by a doctor from the SMA expert centers. Of the 17 detected, 9 were registered in the Registry, meaning the percentage of missing registrations is 47.06%, although all asymptomatic ones were registered. At the same time, asymptomatic patients will not register in other cases since, in the absence of screening, they will not be diagnosed with SMA. This figure should be added when there is no actual screening data. Efficiency of patient-registry interaction, the average time (days): to diagnose 28.56 (median 25,5), from diagnosis to registration 62.8 (median 33,5).

During 2018/20, the number of registrations of SMA Type I patients (meaning the patient was born, diagnosed, and managed to register in the Registry within year) averaged 8.33, which correlates with the number registered in 2022/2023 (9) over the period 11 months. The calculation of the number of patients, considering data on those unregistered due to the absence of symptoms or willingness, is 16.35. Thus, the analytically derived number of patients that should be registered (~17) aligns with the number of patients detected through the NBS (17).

The comparison of data from NBS over 11 months correlates with the results of inclusion in the Registry. The existing registration system, supported by patients and doctors, is justified, sustainable, and reliable, and can serve as a source of demographic information in the absence of a national NBS.

P75

Large bi-regional newborn screening program for SMA in France linked to a nationwide strategy for standardized treatment and follow-up: Initial challenges and current widespread acceptance

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Newborn screening programs (NBS) for SMA are now in place in many countries worldwide, but the paths used to implement these programs and the treatment decision making process vary widely between countries. Many legal and ethical hurdles have delayed the initiation of a French NBS for SMA, but the centralized procedures and the very inclusive healthcare system have led to propose an original model for SMA NBS closely linked to a national registry and to a nationwide strategy for treatment. We report the experience of the French newborn screening program for SMA (DEPISMA) which has started in 2022 in two large French regions. The aim of the project is to test the acceptability and feasibility of systematic NBS for SMA in France and to share conclusions that may be useful to all.

All babies born in Grand Est and Nouvelle-Aquitaine are eligible for the DEPISMA program. A specific detailed information is delivered to families and the signed consent of both parents is required. The blood sample is taken together with classical Guthrie procedures. The NBS itself is performed in two laboratories (one per region) using a commercial automated quantitative PCR method. Positive cases are discussed in a nationwide multidisciplinary commission. The main endpoints are the exhaustivity of the screening and the time until treatment. Other endpoints include economic evaluation and qualitative assessments of the procedure by healthcare professionals and families.

All maternity units in the two regions have agreed to take part in the DEPISMA project (n=81, 100%) and 84% of them are actively recruiting (n=70, accounting for 91% of the births in the two regions). The program has included 31,207 newborns at the time of this abstract and currently includes a mean 1,508 newborns per week. The overall exhaustivity rate of the NBS reaches 92% of the births in the recruiting sites. The acceptance rate by parents is above 99.2%. The incidence of SMA cases found so far is 1/15,600. Two positive cases have been identified at the time of this abstract. The first patient has 3 copies of SMN2 and has been treated by gene therapy at 27 days of age. The second patient has 4 copies of SMN2 and is being closely monitored by electromyography. All positive cases are discussed in a nationwide procedure and included into the national registry for systematic follow-up. No false positive or false negative has been found so far.

The DEPISMA program has adapted a NBS procedure for SMA to the French context after the last update of national bioethics laws in 2021. It proposes a global strategy linked to a national registry and a nationwide treatment commission. Despite initial legal and ethical challenges in France the acceptance of the whole procedure is very good among healthcare professionals and families. It should pave the way to the rapid implementation of a nationwide program possibly including other rare genetic diseases on the same basis.

P76

The 11 months experience in neonatal screening of SMA in Ukraine

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Spinal muscular atrophy (SMA) is an autosomal recessive inherited disease characterized by progressive muscle atrophy and weakness due to the degeneration of spinal motor neurons. The main cause of SMA is a deletion in the *SMN1* gene. The SMN2 is a highly homologous copy of the *SMN1* gene and produces a small amount of functional SMN protein. An increase in the number of copies of the *SMN2* modifies the disease phenotype. Without treatment, children with SMA type 1 do not survive to two years of age. The implementation of neonatal screening (NBS) for SMA is already spreading around the world. In October 2022, expanded neonatal screening for 21 rare diseases, including metabolic disorders, SMA, and SCID, began in Ukraine.

The 11-month results of NBS for SMA in Ukraine were analyzed.

The neonatal screening for SMA in Ukraine was performed by the RT-PCR analysis of the SMN1 gene ("Biocore® SMA/TKID plus», Ukraine). 7 and 8 exons copy numbers of the *SMN1* and *SMN2* gene were analyzed by MLPA method (MRC Holland).

During 11 months of work, 127,400 newborns were screened, as a result 17 children with SMA were identified. The common frequency of SMA is 1 to 7500 newborns in Ukraine is established. The analysis of regional diversity of SMA frequency is complicated now because of the intensive relocation of inhabitants in Ukraine due to the war. The largest group of patients (7 patients, 41%) had 3 copies of 7-8 exons of the *SMN2*, 5 patients had 2 copies, 3 patients had 4 copies and one patient each had 1 and 5 copies of 7-8 exons of the *SMN2*.
The newborn screening is free for all babies born in Ukraine. The entire process of neonatal screening is monitored and recorded in the electronic health care system: from the registration of the newborn and the taking of blood samples by the doctor to the processing of the referral by the laboratory technician and the recording of the diagnostic report based on the research results. Today, two drugs for the treatment of SMA are available in Ukraine - Risdiplam, and Nusinersen.

The expansion of the neonatal screening program and digitalization of processes will make it possible to identify the risks, begin timely treatment of SMA in infants for prevention of their clinical manifestations as soon as possible, and creating conditions for a long and fulfilling life for patients.

P77

Impact of document sharing and training program in the field of SMA Newborn screening

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Treatments for spinal muscular atrophy (SMA) have changed the course of the disease, especially in patients treated before the symptoms appear. Newborn screening (NBS) allows the earliest possible identification of affected children. Several studies have demonstrated the dramatic effect of treatment delivered in the first days of life. NBS programmes are developing rapidly around the world. We set up a pilot project in Southern Belgium (March 2018 - March 2021), which allowed us to gain hands-on experience on the organization and the clinical and psychological management of patients identified by NBS.

Since the start of this pilot project, we have shared all the implementation tools in open access. The entire Belgian application was shared with 34 teams from 24 countries between Marsh 2018 and June 2023. We also developed the NBS Academy, which was designed to enable to share NBS experience of first pilot programs and to promote direct exchange and discussion about difficult cases. The two sessions were attended by 106 participants (45 on site and 61 online) from 42 countries. Satisfaction questionnaires were carried out after each session of NBS Academy, revealing the excellent level of satisfaction of the participants: overall, 92% of participants rated the event as "excellent" and 8% as "very good".

The objective of this research is to evaluate the real impact of data and knowledge sharing of the pilot through direct application sharing and educative events on the expansion of newborn screening worldwide. In this aim, we created two questionnaires to find out if the sharing of the pilot application and/or the attendance to the NBS Academy had any impact on the development of pilot projects worldwide. The surveys were distributed to the 34 PIs who requested access to the pilot application and to the 106 attendants of the NBS academy. We will present the results of these surveys during the meeting.

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Spine MRI as potential biomarker of disease progression in SMA patients treated with Nusinersen: A pilot study

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Nusinersen is a recently approved treatment for spinal muscular atrophy (SMA) that appears to prevent the degeneration of alpha motor neurons (αMN) in the spinal cord (SC), slowing the progression of the disease[1]. Quantitative magnetic resonance imaging (MRI) of the cervical spine has been widely demonstrated to detect and quantify spinal cord atrophy, particularly gray matter atrophy, resulting from motor neuron and white matter degeneration[2,3,4].
The aim of our study is to introduce new potential MRI biomarkers to evaluate the efficacy of Nurinsen treatment in halting or delaying motor neuron degeneration, focusing on differences between adults treated at advanced stages of the disease and infants treated at earlier stages.

Three adult and five infant SMA patients under treatment with Nusinersen underwent longitudinal clinical (HFMSE)[6] and MRI examinations from baseline to 36-month follow-up, with 6 timepoints (T0, T8, T12, T24, T36 months). The MRI protocol included a 3D T2w and T2*GRE sequence centered on the cervical spine. Total spinal cord area (tCSA) and gray matter area (gmCSA) were automatically segmented at the C3-C4 level using STC (Spinal Cord Toolbox)[5]. We then performed a longitudinal assessment of clinical and imaging outcomes in SMA patients, comparing the progression tCSA and gmCSA over time between adults and infants who received earlier treatment.

Patients' motor function remained relatively stable in adult patients, whereas an improvement in motor performance has been detected in the pediatric cohort.
Regarding the MRI evaluation of the SC, all adult patients showed a slight, non-significant increase in total cross-sectional area (tCSA) at TP36 compared to baseline (meanCSA=+0.423mm^2; p=n.s.). Two of the three adult patients showed a slight decrease in gray matter cross-sectional area (gmCSA) at TP36, whereas one patient exhibited a subtle increase in gmCSA, but without reaching statistical significance in either cases, indicating substantial stability.
In the pediatric population, global tCSA showed a slight, not significant increase (ΔCSA=+1.150mm2; p=n.s.) at TP36 compared to baseline. GmCSA statistically significantly increased in all patients at the last timepoint (meanCSA=+0.42mm^2; p=n.s). It is worth noting that during childhood and adolescence, the spinal cord and subsequent tCSA and gmCSA increase due to the physiological growth of the spinal cord. Therefore, at this stage of the study, we can only hypothesize a potential beneficial effect of Nurinsen in preventing motor neuron degeneration in the cord gray matter.

In this study, SC qMRI suggests a higher response to Nurinsen in infants who received therapy significantly earlier than adults, with greater improvement in motor function and apparently regular spinal cord growth, reducing total spinal cord and gray matter atrophy. Further studies on larger cohort are necessary in order to confirm these findings.

P79

Benchmarking care for adults living with spinal muscular atrophy (SMA) in Europe: A call to action from SMA Europe

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Individuals living with SMA require complex care from a multidisciplinary team of healthcare professionals. Recommendations on standards of care (SoC) in SMA were updated before pharmacological treatments were widely available and are primarily focused on paediatric patients - though calls for updates have been made.
In partnership, F. Hoffmann-La Roche Ltd and SMA Europe conducted a benchmarking assessment to understand how care is provided for adults living with SMA in 23 European countries, to identify gaps and best practices. On the basis of these results, SMA Europe is now calling for action to address critical gaps and to improve the provision of care and quality of life for adults living with SMA.

The assessment was conducted in three phases:

- Phase 1: with the guidance of an external advisory group (EAG) of patients and healthcare professionals, reaching consensus on data collection methodology and benchmarking indicators.
- Phase 2: Collection and analysis of data from published sources; a structured survey of clinical experts, and semi-structured phone interviews with patient organisations.
- Phase 3: Integration of results, summarization of key insights, key gaps, best practices, and rating against predefined indicators.

Based upon these findings, and the advice of the EAG, a European wide call to action, inclusive of key recommendations, is proposed.

Herein we highlight the key areas for action and policy change identified and prioritised by SMA Europe, following the benchmarking analysis.

These results and recommendations can be used by healthcare professionals, patient advocacy groups and policy makers to identify areas for action and catalyse improvements in the provision of care and quality of life of adults living with SMA. While this poster focuses on common needs and opportunities within Europe, country specific reports will also help identify the individual and nuanced needs of a national SMA health and social care system.

This study is funded by F. Hoffmann-La Roche Ltd, Basel, Switzerland and was conducted in partnership with SMA Europe. Data collection and analysis were conducted by Hall and Partners, UK, and Weber Shandwick, Belgium, respectively. Data collection, analysis and medical writing support are funded by F. Hoffmann-La Roche Ltd.

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P80

Characterising access to care management for adults living with SMA in the UK through Adult SMA REACH Data collection study

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Adult SMA REACH, a national clinical network and data collections study, aims to characterise adults living with Spinal Muscular Atrophy (SMA) in the UK, understand the impact of new treatments on the natural history of SMA and standardise care provision. SMA is a form of motor neuron disease caused by a mutation in the survival motor neuron 1 gene (SMN1) which results in a wide disease spectrum affecting infants and adults.

The recent advances in drug development and European Medicines Agency approvals of Disease modifying therapies (DMT) highlight the need for standardised, longitudinal, real-world data collection across this emerging cohort and timely access to care provision.

Using the Adult SMA REACH dataset we aim to establish how many and which patients have access to specific aspects of care provision.

The dataset comprehends 370 patients across 19 sites in the United Kingdom, with over 1200 patient visits at the time of writing.

Data from adult patients enrolled in the Adult SMA Reach project will be analysed. Data includes validated functional outcome measures, reported care management and access to care interventions. This includes physiotherapy provision, use of mobility aids, respiratory and cardiac care. We will categorise patients according to their SMA type, mobility status and treatment. Using this strategy, we will be able to describe access to care intervention and provision for adults living with SMA in the UK using the Adult SMA Reach data set.

Cross-sectional analysis is ongoing and will report on access to care management and its impact on disease severity.

Due to the current availability of Disease Modifying Treatments, and improved care standards, there is an opportunity to identify gaps in care provision and inequality in access to care and treatment. Ultimately this project will help in providing better care for adults with SMA in the UK.

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The effect of online grief consultancy program (e-PsychoYDP) in bereaved mothers of children with spinal muscular atrophy

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The grief of loosing a child is a huge trauma and may adversely affect individuals. The reactions such as not being able to get over the mourning process and suppress the grief course can cause chronic pathological problems which cause depression and psychosomatic diseases. In the national literature, there is no study regarding intervention programs which aims enforcing bereaved mothers of children with spinal muscular atrophy provides and provides healthy overcoming the mourning process.

The aim of this study is to evaluate the effect of the online grief consultancy program (e-PsychoYDP)- which will be given to bereaved mothers- in terms of grief process, psychological wellbeing and quality of life. In recent years, internal applications, which are close to face -to -face, are more preferred. Since e-PsychoYDP is free of charge, easy to access and participants can get more comfortable service in the environment in which they feel safe, it is considered that more participants will demand to participate in the program and it is planned in online format.

This study will be conducted in a recurrent measured experimental research design, including a pre-test, post-test and control group. Target group will be accessed via the website to be created. Applicants who registered through the website will be informed by a psychiatric nurse involved in the research and after that they would be invited to the study. Mothers who wish to be included in the program will be appointed to experimental and control groups respectively.

Personal information form, mourning scale, psychological wellbeing formation scale and quality of life scale will be applied to the experimental and control groups. Then, online attempt will be made with e-PsychoYDP, which consists of 8 sessions (8 weeks) for the experimental group and lasting for 60- 90 minutes each. Experimental groups will be taken into sessions in groups of 5. Online sessions will be held with the mothers in the control group twice (4th and 8th weeks) lasting 10- 30 minutes, allowing the researcher to interact. In the interviews with the control group, information will be given in line with the education needs of the mothers. Once the e-PsychoYDP sessions of the experimental group are completed, follow-up tests will be applied to both groups (experimental group & control group) through online interviews, after one and three months respectively.

As a result of the research, it is expected that the intensity of grief will decrease and the psychological well-being and quality of life will increase in bereaved mothers by the help of e-PsychoYDP. In addition, it is thought that the data to be obtained will be a guide in the creation of intervention programs for bereaved mothers of children with spinal muscular atroph.

It is thought that this study will allow health professionals to early recognize the reactions of the bereaved mothers of children with spinal muscular atrophy in the mourning process. So that appropriate health services and rehabilitation methods can be applied.

P82

Ethical considerations to address when treating patients with spinal muscular atrophy: The Nordic Experience

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In the era of newborn screening and access to gene therapy, one could argue that the ethical issues surrounding the detection and treatment of SMA begin to fade. How is the ethical framework changing along with the changing landscape of SMA detection and treatment and how does this correlate to providing patient-centered care?

A Nordic scientific roundtable discussion and subsequent meetings took place in 2022 to capture and share the experience of Nordic countries, i.e. Sweden, Norway, Finland and Denmark, regarding the outcome measures used in the evaluation of current treatments for SMA as well as to highlight unmet needs. Participants were mainly healthcare professionals specializing in neurology, pulmonology and physiotherapy with expertise within the field of SMA. Here, we present our initial consensus results and recommendations.

Four major topics were highlighted, i.e. (a) unequal access to targeted therapies; (b) complexity of the therapeutic decision-making; (c) stress and fear of losing access to ongoing treatment; (d) uncertainty about the evolution of the disease and associated co-morbidities. Establishing treatment benefit was the common denominator in many of the ethical issues discussed. The role of functional assessment tests in showing treatment benefit is major, making patient performance on these tests a great source of anxiety for the patient and the family. New survivors and older patients with SMA constitute a previously unknown group of patients with a new panorama of co-morbidities. This disease evolution inevitably poses new challenges to the evaluation and decision-making process.

There is a need for open and clear communication between the clinicians and the patient/family to acknowledge the ethical challenges arising with the evolution of the disease and involve the patient in his/her care. Harmonizing the criteria for patient identification and access to high-cost therapies is essential. Further work is needed to increase awareness regarding the therapeutic decision-making, to ensure informed decisions and patient-centered planning of care and rehabilitation.

P83

Physiotherapeutic management of patients with SMA: A questionnaire-based online survey among physiotherapists within the SMArtCARE registry

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With changing phenotypes under disease-modifying treatment, adaptions in the physiotherapeutic management of patients with spinal muscular atrophy (SMA) are strongly required. The object of this study was to map the physiotherapeutic management of patients with SMA within the SMArtCARE network. SMArtCARE is a disease-specific registry for patients with 5q-SMA with 70 participating centers in Germany, Switzerland, and Austria. Physiotherapists are regularly trained in workshops and webinars.

An online survey among physiotherapists using the Delphi method was conducted with two questionnaire rounds between June/2022 and June/2023. Seven experts developed and revised the respective questionnaires. The second questionnaire was based on eight case studies reflecting different levels of motor and respiratory function. Questions referred to four thematic blocks: stretching, positioning, muscle strengthening, and respiratory management.

The second questionnaire was sent to 148 participants and there was a return of 44 responses of which 41 responses were evaluable. This corresponds to a survey response rate of 27.7%. 31% were physiotherapists from neurologic Dept.s and 65.9% were physiotherapists from neuropediatric Dept.s. Most of physiotherapists were well experienced in treating SMA patients (experience >3 years with >7 SMA patients). The majority of respondents perceive a need to perform stretching exercises in all SMA patients with existing contractures (75%-95.65%) and still, one third of the participants consider stretching exercises necessary even with free joint mobility (29.41%-33.33%). Customized seating was recommended for non-sitters, sitters, and walkers with limited walking ability (72.22%-91.30%). Supported standing exercises were considered for all sitters and non-sitters with sufficient head and trunk control (66.67%-91.30 %). The majority of participants used muscle strengthening training in all SMA patients (60%-100%) and agreed that a neurophysiological-based concept is needed in the therapy of SMA patients (84.62%-100%). Further, there was a consensus that prophylactic respiratory management is necessary in all SMA patients with weak cough (81.25%-100%).

We here present an overview of the physiotherapeutic management and recommendations derived from physiotherapeutic experts from the SMArtCARE network that might contribute to developing and advancing guidelines for the physiotherapeutic treatment of SMA patients.

Access

P84
OdySMA – A quest to access: An SMA Europe advocacy tool
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SMA Europe aims to improve access to diagnosis, optimal treatment and care for all individuals in Europe living with spinal muscular atrophy (SMA). However, access to healthcare differs substantially across the spectrum of all individuals living with SMA as well as across European countries. Therefore, SMA Europe members have requested support to better understand the European situation, identify hurdles to gain and maintain access, and initiate advocacy and policy shaping initiatives to overcome them. SMA Europe addresses these needs by collecting data supported by other stakeholders to gain a full overview over the access situation in different European countries, and by bringing together all relevant data onto one online platform.

"OdySMA – a quest to access" is an initiative with the following objectives: 1) to map the situation regarding access to diagnosis, treatment and care in all SMA Europe member countries; 2) to help understand access differences across countries and the spectrum of individuals living with SMA, identify access priorities, gain the required access skills and build successful advocacy strategies to improve access to treatments and care; and 3) to inform research priorities.

Five defined sections describe key policy components for improving access to SMA diagnosis, treatment and care: 1) SMA health care ecosystem; 2) SMA diagnosis; 3) access to SMA medicines; 4) access to SMA treatment other than SMA medicines; and 5) access to SMA care.
Results show that there are inequalities around access to medicines across countries and spectrum regarding e. g. SMN gene copy numbers, weight or age. But also access to newborn screening for SMA is not equal in all member countries and sometimes even differs within a single country by region. In general, the field around health services supply for SMA is evolving and therefore the platform needs regular updates.

The outcomes are used to inform patient advocates as well as relevant European decision-makers on patients' unmet needs regarding access. Moreover, results form the basis for establishing strategies to counteract identified access hurdles and health inequalities in advocacy trainings for SMA Europe member organisations. Also, OdySMA builds capacity in the wider community to strengthen the position of patients in the healthcare ecosphere.

To make statistics more understandable, to show the impact of the numbers on the life of people living with SMA, and to facilitate action within the respective stakeholders, SMA Europe works now as a next step on humanizing the data by adding to the quantitate a qualitative data layer by sharing personal stories that highlight remarkable data points as well as to illustrate gaps in knowledge and data.

P85
Medical Crowdfunding in Spinal Muscular Atrophy (SMA): A modern tool to fight for survival?
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In SMA, some families are facing the situation of not having access to specific medicines through their healthcare system and the financial burden of paying for the medicines privately is too high. Therefore, medical crowdfunding seems to be a strategy to access medicines. With the launch of medicines, particularly Zolgensma, the crowd-funding activities in the SMA community have reached a peak.

In this study, we discuss the factors that lead families to pursue this avenue to access medicines, and we highlight the impact on families and the challenges they are confronted with. Finally, we map the crowdfunding initiatives in SMA over the recent years.

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From Possible to Accessible - Taking the final step in the SMA patient journey

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SMA is now identifiable, preventable and treatable. That doesn't mean anything unless testing and treatment are also available, affordable and accessible. Making this a reality requires a partnership approach from health professionals, industry, governments, media, patient advocacy leaders, patients and families. How can this happen?

The first steps requires mindful collaborative intent, followed by the intentional establishment of objectives and an understanding of the environment to create a new generation SMA for a country. Achieving these outcomes is a marathon not a sprint and beyond these activities, needs commitment, purposeful activity and action, real world evidence based data and a plan that enables momentum for the required change.

This focussed intent will be captured in a co-designed strategic action plan. Identifying what is known, what evidence is needed and what exists, who is involved and in what capacity, directing experts, advocates and evidence at key decision makers are requirements for a successful outcome.

Success can be measured in equitable and affordable access to testing, treatment and care. Are patients and family's lives being improved by the potential we now have? In Australia, the answer is yes, the work was pioneering and exhausting.

The SMA Australia case study delivering genetic carrier testing, newborn screening, and access to all existing SMA treatments(from pre-symptomatically right through to adults) for the current and future SMA community captures the elements of discovery to implementation. This is the result of a structured, strategic, and deliberate collaborate effort that delivers results that impact each step along the patient journey.

Developments in genomics and other 'omic' technologies continue to be exciting and have revolutionised our choices in therapy and care practice. Having the capability within our health practice doesn't necessarily lead to implementation, we have seen and experienced this over and over when a conscious implementation plan isn't in place. Capability doesn't mean capacity and doesn't mean choice and access. And if they aren't there, what's the point.

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Difficulties of the Spanish adult population to access treatment: SMA Registry in Spain

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Over 95% of individuals living with SMA present symptoms during their paediatric years. This phenomenon could account for its classification as a childhood illness, consequently leading to a lack of understanding regarding the disease's impact and challenges in adults, making them a less visible group. For some authors, adults constitute up to two-thirds of the total SMA-affected population
In the Spanish National Registry, RegistrAME, half the patients (164 individuals) are aged 16 years or older, spanning all age groups up to 77 years (m: 34)
Adults bear the burden of years of physical decline, resulting in significant physical disability and dependence. RegistrAME data reveals that 32% of adults are non-sitters and 50% sitters. Among the 18% classified as walkers, some also experience significant limitations, preventing them from achieving functional gait. Also, one in four adults either lacks useful hand function or, if they have it, cannot reach their mouth

Starting in 2018, adults gained access to the first disease-modifying treatments (DMTs) in Spain, specifically nusinersen. Nevertheless, access had been restricted as 44% of the adult population remained untreated at the time of Risdiplam's approval in December 2022. However, this milestone did not bring the expected significant change, as one in every three adults still does not receive any DMT despite the profound impact of this disease. This contrasts starkly with the paediatric group, which maintains a 99% treatment access rate

Nusinersen treatment was interrupted in 11% of adults as they did not fulfil the improvement criteria established by Spanish reimbursement authorities. Following withdrawal, and after at least eight months, during which deterioration was evident due to the lack of treatment, some patients could resume treatment. However, this was not the case for 13 patients, who, following discontinuation for various reasons were unable to restart treatment with any of the approved therapies

Adults experiment important difficulties regarding inclusion in clinical trials or compassionate-use programmes. In addition, they are more vulnerable to receiving a treatment discontinuation decision because the criteria they must fulfil to continue treatment are based on the efficacy results of clinical trials performed on younger subjects. Regardless of the availability of two DMTs for adult SMA patients, a substantial constraint in accessing these therapies persists, despite the evident impact of the disease on this group. This becomes particularly concerning for those with a high degree of disability, who face an imminent need to prevent further deterioration of their motor functions
A greater visibility of adult patients, the impact of the approved DMTs, and their unmet needs is essential. Adult patients experience significant deterioration, lower access to marketed treatments, and very limited access to clinical trials

Patient perspective

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"Not only has she survived, but she lives a happy life" - A nationwide study on parents' perspective on their child's treatment.

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Until recently no treatment has been available for patients with spinal muscular atrophy, (SMA), but now several treatment options have been introduced and probably more will come. The new treatments have significantly changed the life and prognosis for the ill, but also the scenario for families. In particular quality of life for children with SMA 2 has improved according to validated scales. Yet, they fail to fully reflect the child's abilities as perceived by the parents. Moreover, knowledge about long-term improvement and side effects of the treatment is sparse.

The aim of this study was to explore parents' view on the information given about new treatments and their potential effects on child's capabilities over time.

This prospective investigation was based on a nationwide cohort using Swedish national registers to identify children with SMA who had been cared for as an in-or outpatient at any time during 2018 and their parents. Four consecutive electronic questionnaires were sent to parents, i.e., every 6 months, over two years, from September 2020 to March 2022. The study was approved by the Ethics Review Board in Stockholm. Data were analysed using descriptive statistics and content analysis.

Parents of 33 children participated. The first questionnaire was filled out by 47 parents, the second by 38, the third by 40 and the fourth by 37. All parents had agreed to Spinraza treatment of their child. Given the experience of treatment, all except two parents were positive and would make the same decision again. A majority of parents (89%) felt they had received adequate information about treatment. Over time 137 free hand comments were provided by the parents on the child's capabilities. Most of these reported that the child had become stronger, more energetic, and independent after each treatment round.

A vast majority of parents of children with SMA remain positive to Spinraza treatment and the information given.

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Identifying the top 10 unanswered research priorities for spinal muscular atrophy

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Health research agendas are usually drawn by researchers or by industry and may not reflect the needs and priorities of end users. This priority-setting partnership (PSP) for spinal muscular atrophy (SMA) was undertaken to identify the most pressing unanswered questions about SMA from the perspective of people with SMA, their carers and health and social care professionals.

Using the methodology developed by the James Lind Alliance (JLA), evidence uncertainties were gathered online, using Survey Monkey®, from people with lived experience of SMA, including their carers, family members and health and social care professionals across Europe. The survey was translated into 17 languages and distributed through our stakeholder networks, as well as those of our member countries and beyond. Non-clinical researchers and employees of the pharmaceutical were excluded.

A total of 915 participants over 22 countries answered the survey, providing 1,337 responses. Of the participants that asked at least one question, 45% live with SMA, 36% are either carers or family members of people living with SMA and 19% are health and social care professionals. All types and age ranges (0 to 65+) were represented, with the majority being Type 2 (47%), followed by Type 3 (31%) and Type 1 (14%). The balance was made-up of a pre-symptomatic individual, a person living with non-5q SMA and people who did not divulge their SMA Type. Health and social care professionals included neurologists, paediatricians, physiotherapists, nutritionists/ dieticians, speech and language therapists, psychologists, occupational therapists, respiratory clinicians, geneticists, nurses and social care workers.

The next phases of this PSP will see the categorisation of the questions to produce a list of summary research questions. Those already answered in the existing literature will be removed. In a second survey, participants will be asked to rank the summary research questions in order of priority. An as yet undetermined numbers of unanswered research priorities will then be considered during a workshop to agree on a 'top 10'. Ultimately, these top 10 unanswered research questions will be disseminated widely to researchers and funders.

This project represents the first systematic evidence of patient and clinician-centred unanswered research priorities for SMA. The results of this priority-setting

exercise will provide an opportunity for researchers and funding agencies to align their agendas with the needs of the SMA community, in order to improve clinical outcomes and quality of life.

P90

The SMA Daily Life Study – An experience sampling study examining interrelations between patient-centric outcomes in the daily lives of individuals living with SMA

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Patient-relevant outcomes such as fatigue, pain and mental health are getting more and more research attention because they seem to play an important role in the general well-being of people living with SMA, and because they might be valuable as outcome measures for new treatments. Several attempts are currently being made to develop instruments to both subjectively and objectively assess such outcomes. However, it remains unclear how these constructs relate to each other both at the between-person and at the within-person level. This poster contributes to gaining a better understanding of the relationships between different patient-centric outcomes by assessing them dynamically, in the actual daily lives of individuals living with SMA, by utilizing data from the SMA Daily Life Study.

The SMA Daily Life Study included 39 participants living with SMA from sixteen European countries. This study used the Experience Sampling Method (ESM) by prompting participants to respond to an electronic questionnaire through the smartphone application m-Path, six times a day for ten consecutive days. On average, participants reported to 76.9% of the prompts. The SMA Daily Life Study included psychological (e.g., emotions and self-esteem), psychosomatic (e.g., pain and fatigue), social (e.g., interactions with others), and contextual (e.g., location and work) variables. By conducting network analyses, we assessed how these variables related to each other.

Preliminary analyses provided insight into the complexity of relations between different patient-relevant outcomes. For instance, finding it more difficult to move was found to correlate with difficulties in swallowing and talking, but not in breathing. Moreover, fatigue was related not only to being tired, but also to experiencing pain. Finally, being in an accessible environment was related to being able to manage emotions and work better, feeling in control and more confident.

The results from this study show that many patient-centric outcomes are related to each other concurrently. Future analyses will investigate predictive relationships between patient-centric outcomes, to identify variables that are more central to an individual’s experience.

P91

Tracking the presentation of fatigue in daily life for people living with SMA through the Experience Sampling Method

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Fatigue is a key patient-relevant outcome in SMA and numerous efforts are underway to better measure fatigue subjectively and objectively. However, it remains unclear how fatigue manifests in the daily lives of individuals living with SMA. Like other (psycho)somatic symptoms, fatigue may be a stable component in individuals' lives, but it may also show large fluctuations throughout the day. Moreover, individuals living with SMA may differ from each other in how their fatigue presents, in the causes of their fatigue and in how they recover from it. This poster contributes to gaining a better understanding of fatigue by assessing it dynamically, in the actual daily lives of individuals living with SMA, by utilizing data from the SMA Daily Life Study.

The SMA Daily Life Study used the Experience Sampling Method (ESM) to collect intensive longitudinal self-assessments from a sample of 39 people (mean age = 36 years) living with SMA. Participants were prompted six times a day for ten consecutive days to respond to an electronic questionnaire through the smartphone application m-Path. On average, participants reported to 76.9% of the prompts. The study included variables for fatigue as well as psychological (e.g., emotions and self-esteem), psychosomatic (e.g., pain), social (e.g., interactions with others), and contextual (e.g., location and work) factors.

Results show that there is not only a large amount of between-person variability, but also a large amount of within-person variability in how fatigue is experienced. Individuals do not only differ from each other in their fatigue experience, but their fatigue also considerably fluctuates over time. Moreover, participants conceptualized fatigue in different ways; for some, it was mainly an indication of being physically tired, whereas for others, mental tiredness also played a role. Finally, individuals differed in how they recovered from their fatigue, some recovering more quickly than others.

The results from this study show that the experience of fatigue is very complex, with both between- and within-person differences in quantity but also quality of fatigue. This implies that to really understand and measure fatigue in a meaningful way, a more personalized, idiographic approach is needed.

POSTER SESSION 2

FRIDAY 15 MARCH

16.00 - 17.30

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MAP THE SMA protocol: A Machine-learning based Algorithm to Predict THErapeutic response in Spinal Muscular Atrophy

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In recent years, there have been remarkable strides in SMA treatment, with three globally approved disease-modifying drugs. Yet, the role of various clinical variables in shaping the overall SMA phenotype remains poorly understood. This study employs an innovative machine learning-based approach (ML), aiming to develop a predictive algorithm using data from SMA patients.
Methods. The study underway aims to gather data from patients with SMA, including those afflicted with SMA I, II, III, and presymptomatic cases, whether or not they have already received treatment.
The data collected will consist of clinical, biological, and Patient Reported Outcome Measures (PROMs) data. The clinical data will include information about patients' demographics, medical history, neurological assessment, motor function measures, and laboratory results. PROMs will be collected using standardized questionnaires to assess patient-reported outcomes, such as the PEDI-CAT, SMA-HI, and others. Additionally, the RNA molecular signature profiling will be carried out using Next-Generation Sequencing to identify possible novel biomarkers.
ML algorithms are proving to be useful tools in predicting individual responses to treatments and interpreting outcomes. By leveraging the power of ML, researchers hope to identify patterns and associations within the large amounts of data being collected, leading to new potential treatment approaches.

As of August 17th, 2023, we have retrospectively collected data from 70 SMA patients, comprising 8% pre-symptomatic, 27% SMA I, 38% SMA II, and 27% SMA III cases, all treated with nusinersen. This data was originally collected from February 9th, 2022, to April 29th, 2023. Among these patients, 51% are female, and the distribution of SMN2 copy numbers is 34% for 2, 50% for 3, and 16% for 4+ copies. Data on Creatinine, circRNA, functional status, outcome measures, scoliosis status, Cobb Angle, scoliosis surgery, contractures, ventilatory status, nutritional status, anthropometric measures, and BMI have already been collected for these patients. Additionally, 70% of this cohort has at least one PROM at one data point (SMA-HI, center-specific questionnaire, Orsat level). Furthermore, we are enrolling patients in the prospective data collection phase of the study. In addition to the variables described above, data on Orsat checklist and PEDICAT have been gathered for this cohort.

The study underway offers a novel approach to SMA research through the use of ML. By analyzing large amounts of data, researchers can identify patterns and associations, leading to personalized treatments that cater to the specific needs of each patient. The ML algorithm offers a significant shift towards personalized medicine, ultimately improving outcomes for patients and enabling more efficient use of healthcare resources.

P93

Time to lose ambulation in an untreated cohort of patients with type III SMA: results from the Italian ISMAC registry

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Previous research on time to ambulation loss (LOA) is limited, with notable studies by Zerres in 1997 (Germany and Poland), Brian H.Y. Chung in 2004 (China), and Anna Lusakowska in 2021 (Poland). Understanding the timing of ambulation loss is vital for optimizing patient care and evaluating future treatments, especially considering the rarity and complexity of SMA. Additionally, this data would provide a fundamental reference point for comparing treated patient outcomes and assessing the effect of therapies (DMTs). Utilizing data from the Italian ISMAC registry (ITASMAC), this study aims to identify LOA risk factors.

Data were collected from ITASMAC for both pediatric and adult SMA Type III patients up to the initiation of DMTs. The primary objective is to compute the risk of ambulation loss, employing statistical methodologies such as Cox proportional hazards models and Kaplan-Meier survival analysis. Additionally, for the exploratory aim, time-dependent Cox regression was utilized to evaluate the influence of DMTs on ambulation loss, examining variations among different Type III subtypes.

Data from 404 type III SMA were analysed, featuring an even distribution between subtypes IIIA and IIIB. Gender distribution was balanced, with 47.5% females. SMN2 copy number analysis revealed that 39.6% had 3 copies, 35.9% had 4+ copies, while only 7.7% had 2 copies, and 16.8% had unknown copy numbers. Diagnoses were categorized into three periods: pre-Standard of Care (SoC) 2007 (44.6%), SoC 2007-2017 (29.0%), and post-SoC 2017 (23.5%). The median age of LOA in the entire cohort was 36 years.
Patients with 4+ SMN2 copies exhibited a significant reduction in the risk of LOA with a risk reduction of 59% ($p < 0.001$). Sex or era of SoC did not show significant risk reduction. Additionally, when comparing IIIA and IIIB patients, IIIB had a significantly reduced risk of LOA (78% ($p < 0.001$)). The median age at LOA varied significantly between the two subtypes, with IIIA losing ambulation at a median age of 13.6 years, while IIIB at 48.0 years. Importantly, the combination of subtype (A/B) and SMN2 copy consistently differentiated IIIA and IIIB patients.
When comparing untreated patients to those who received DMTs (exploratory analysis), out of 159 treated patients, only 10 (6%) experienced LOA during treatment, specifically, 4 IIIA and 6 IIIB (range DMTs duration 1-7 years). From a preliminary analysis, treatment acted as a modifier for IIIA ($p = 0.02$). However, it is important to acknowledge that the follow-up duration remains relatively short for a comprehensive evaluation.

This study, utilizing data from the Italian ISMAC registry, provides valuable insights into the natural history of Type III SMA and factors influencing LOA. Extended follow-up research is needed to validate these findings and explore DMTs effect for Type III SMA patients.

P94

Contemporary analysis of the Australian clinical and genetic landscape of spinal muscular atrophy – A registry based study

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Disease modifying therapies for spinal muscular atrophy (SMA) have significantly changed disease course and management. Within this moving landscape, registries are a critical tool to increase knowledge, monitor interventions and support translational research to improve quality of healthcare and guide service provision.

To describe the contemporary clinical and genetic landscape of SMA in Australia through the national registry.

This cross-sectional study included all individuals with a genetic diagnosis of 5q SMA enrolled in the Australian Neuromuscular Disease Registry (ANMDR) from 2020 to 2023. Demographic, clinical and genetic data was collated and analysed using descriptive statistics.

As of June 2023, 195 individuals with SMA were enrolled in the ANMDR (56% children, 44% adults; age range 0.6-75 years, 51% female). Five were deceased. There was a phenotypic spectrum, with 1 SMA0 (0.5%), 45 SMA1 (23%), 76 SMA2 (40%), 65 SMA3 (33%) and 2 SMA4(1%) and 6 presymptomatic/clinically silent (3%). The minimum Australian prevalence of SMA was 0.73/100,000 individuals and reduced with increased age. Diagnosis was through newborn screening (5.1%), family screening (2.6%), prenatal screening (1%) or following symptoms/clinical assessment (91.8%). *SMN1* pathogenic variants included homozygous deletions of exon7 (91.7%), compound heterozygous deletion and sequence changes (7.1%) and biallelic sequence changes (1.2%). *SMN2* copy numbers were significantly associated with phenotype in homozygous deletions. Comorbidities related to weakness (scoliosis, contractures, impaired mobility) were frequent. Disease modifying therapies were prescribed for 96% of children and 63% adults. Access to and engagement with multidisciplinary care was significantly higher for children with SMA than adults (paediatric 85%, adults <10%).

The dynamic and evolving diagnostic and therapeutic Australian landscape encompasses a varied population, with substantial morbidities and healthcare needs. Registry data identify the adult population of SMA to be less served under the multidisciplinary umbrella, representing an unmet need.

P95

The Molecular Landscape of SMA in Turkey

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Spinal Muscular Atrophy is an autosomal recessively inherited neuromuscular disease caused by low levels of SMN protein, which is encoded by the two genes *SMN1* and *SMN2* that have a very high homology. Unless splicing is modified, the *SMN2* gene produces ~10% functional SMN protein per each copy of the gene, while *SMN1* produces ~90% functional SMN protein. SMA patients do not have a functional *SMN1* gene; thus, they must rely solely on the *SMN2* gene. Phenotypic differences across individuals within the same SMA type and even among siblings are evident and previous studies have identified several modifier genes and variants. Little is known on the molecular basis of SMA in Turkey, and the aim of this study is to understand the genetic background and the fine structure of the disease in a small cohort consisting of 12 Turkish SMA families.

In the framework of this study, candidate modifier genes and variants were investigated by WES analysis followed by Sanger sequencing. The copy numbers of the *SMN1* and *SMN2* genes, as well as the copy number of *NAIP* exon 5, were identified by MLPA. Gene expression analysis of the common variations will be performed by qPCR.

Out of thirteen cases (Type 1: five; Type 2: two; Type 3: six) (and thirteen family members) subjected to MLPA analysis, the homozygous deletion of the *SMN1* exon 7 and 8 was detected in all cases, excluding one patient with a homozygous deletion of the *SMN1* exon 7 but with a single copy of *SMN1* exon 8. The patient has a hybrid *SMN* gene and manifests a milder phenotype. While all Type 1 cases were found to have 2 copies of the *SMN2* gene, 2-4 copies were identified in Type 2 and Type 3 patients. Either a homozygous or a heterozygous deletion was detected in *NAIP* exon 5.

SMA research is in its golden era with three treatments and an extensive drug pipeline of several clinical trials. However, a big question mark remaining is the disease-modifying factors. In literature, most Type 1 patients were found to have two *SMN2* copies, and the homozygous deletion of the *NAIP* exon 5 was frequently observed. Our preliminary results are in accordance with the previous reports. The age of onset for SMA Type 3 has a broad spectrum, and disease severity is quite variable; thus, divergent *SMN2* copy number distribution can be expected. Analyzing discordant siblings with the same copy numbers of the *SMN2* gene and *NAIP* exon 5 by gene expression and WES analyses is promising in the discovery of modifying factors. These results are encouraging to further explore the largely unknown SMA genetics in Turkey. As the puzzle of SMA is solved over time, a much more hopeful future will await the patients and their families.

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Current evidence about the adults with spinal muscular atrophy in North Macedonia

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Spinal muscular atrophy (SMA) is a genetic autosomal recessive disorder characterized by progressive muscle weakness, decreased muscle tone, and impairment of alpha motor units. Approximately 25% of SMA patients are adults; however, recent advancements in treatment options are increasing the life expectancies of patients with SMA. Data for adults with SMA is lacking given the focus on infant and pediatric patients. As newer treatment options increase the proportion of patients surviving into adulthood, providers should be aware of SMA in adults and the proper management strategies and tools to ensure ideal patient care. This abstract aims to present the most recent evidence on the demographic and clinical characteristics of the adult SMA population in North Macedonia.

Patient hospital files were utilized to collect data from the only reference center in the country for management of adult SMA patients. The retrospective analysis included all diagnosed adult patients.

The analysis included 11 adult SMA patients, with an average age of 37.0 years; range from 17-65 years. The average age of symptom onset was 10.8 years. 54.5% (n=6) of the patients were male. The majority of patients had SMA Type 3, accounting for 63.6% (n=7) of the cases. SMA Type 2 was present in 27.3% (n=3) of the patients, and only 1 patient (9.1%) had SMA Type 4. Regarding motor milestones achieved, all patients (100%) had achieved head control; 45.5% (n=5) were able to walk without support, while 18.1% (n=2) could walk with support, and 36.3% (n=4) of the patients were wheelchair-bound. Genetic analysis revealed that 9.1% (n=1) of the patients had 3 copies of the SMN2 gene, 27.3% (n=3) had 4 copies, and 9.1% (n=1) had 5 copies. The remaining 54.5% (n=6) had an unknown number of SMN2 copies. The average total RULM score for 6 patients assessed was 33.3 (±6.7). Similarly, the average total MFM-32 score for the same 6 patients was 43.1 (±20.2). Out of the 11 patients, 81.8% (n=9) were receiving risdiplam treatment, one patient (9.1%) was eligible for but was waitlisted, and another patient (SMA Type 4) was deemed ineligible for treatment.

Our work has shown a low prevalence of adults with SMA in North Macedonia, but provided valuable insights about their characteristics. The majority of patients were SMA Type 3, and were able to walk without support, while a smaller proportion required support or were wheelchair-bound. The majority were receiving risdiplam, highlighting its importance in managing SMA. Further research is needed to explore the long-term outcomes and effectiveness of the treatment in individuals with SMA.

P97

Demographic and Clinical Characteristics of Patients with Spinal Muscular Atrophy in Kosovo

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Spinal muscular atrophy (SMA) is a rare genetic neuromuscular disorder marked by the progressive loss of motor neurons, leading to muscle weakness and atrophy. Despite its rarity, the impact of SMA is significant. In Kosovo, recent findings have indicated a diagnosis rate of approximately 1-2 pediatric patients per year, underscoring the need for a deeper understanding of SMA's prevalence and clinical characteristics in this region. This study aims to provide insights into the demographic and clinical features of early-onset SMA patients in Kosovo, shedding light on this rare condition's manifestation within the local pediatric population.

An observational, retrospective analysis was conducted using medical records and clinical assessments of SMA patients from the Department of Neurology, Pediatric Clinic at the University Clinical Center of Kosovo. Data were collected during routine patient visits as real-world outcome data.

A total of 6 SMA patients were included in the study, with an average age of 5.4 years (range from 1.3 to 13.7 years). The gender distribution was equal. The average time from birth until genetic confirmation of SMA was long, 23.5 months (range from 2.4 to 64.2 months). SMA Type 1 and Type 2 were equally represented (n=3), while no patient had SMA Type 3. The majority of patients were non-sitters (66.6%), with one sitter (16.6%) and one walker (16.6%). The number of SMN2 gene copies varied, with 50% of patients (n=3) having 2 copies, and the remaining patients having 3, 4, and an unknown number of copies. Three out of five eligible patients (60%) were receiving disease-modifying therapy, two patients were on treatment with risdiplam, and one was on nusinersen. The average total CHOP-INTEND score in two assessed patients was 15.5 (±8.5), and the total HFMSE score in one assessed patient was 8 points.

Our work contributes to understanding the current SMA patient population in Kosovo. The findings emphasize the need for further improvement in obtaining early diagnosis and raising awareness about SMA; funding for access to treatment for all eligible patients; and research on long-term treatment effectiveness. Addressing these challenges is essential for improving individuals' outlook with SMA.

P98

Spinal Muscular Atrophy – New perspectives in the Republic of North Macedonia

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Spinal muscular atrophy is a rare, genetically determined, neurodegenerative, progressive and heterogeneous group of diseases. The responsible gene is mapped on the long arm of the fifth chromosome and in 96% it is a deletion of exon 7 and 8. It is inherited in an autosomal recessive manner, and the dominant clinical manifestation of the disease – muscle atrophy is the result of degeneration of the alpha motor neurons in the anterior horns of spinal cord due to deficiency of the fundamental SMN protein. It is the leading genetic cause of early death.

To describe the current condition of patients with this disease in the Republic of North Macedonia, their genetic architecture, clinical management, current therapeutic possibilities and future perspectives.

Patients diagnosed, clinically monitored and treated at the Clinic for Children's diseases. The method for definitive diagnosis in our country is genomic diagnostics, and patients are monitored multidisciplinary, the effect of pharmacological treatment is determined by applying functional tests.

Currently, in the Republic of North Macedonia there are a total of 20 patients with spinal muscular atrophy, aged from 4 months to 53 years.
At the Clinic for Children Diseases, 12 patients (5 female, 7 male) are being monitored, currently aged from 4 months to 21 years. Based on the age at presentation of the disease, motor achievements, as well as the number of copies of the *SMN 2* gene, in the pediatric group there are 5 patients with Spinal muscular atrophy type 1, and the rest (7) are type 2.
10 of the pediatric patients have biallelic disruption in the *SMN1* gene, and in two patients the cause of the disease is double heterozygosity in the *SMN1* gene.
All patients in our country are treated with genetic modifying therapy and are monitored multidisciplinary. Neonatal screening for this disease is not available.

Key words
Spinal muscular atrophy, genetics, therapy, neonatal screening.

P99

Profiling neuroinflammatory markers in CSF in response to nusinersen treatment in pediatric SMA patients

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Life with spinal muscular atrophy (SMA) is a constant balance between independence and everyday risks such as assistive-equipment failures, wheelchair accidents, and so forth. Seemingly harmless activities of daily living can quickly lead to emergencies (respiratory distress during sleep, for example) if people have no tool to help or to call for help.
In this poster, we present an ongoing study leveraging digital health technologies (non-invasive ambient sensors, wearable devices, and mobile apps) to make people with SMA safer and more independent.

The aim is to develop and test technologies to monitor some of the most significant risks of emergencies as well as more subtle health degradations, and to allow patients to react by calling for help and by sharing insights from key health measures and digital biomarkers with caregivers, to initiate prevention efforts.

As patients shared with us during pre-study interviews including caregivers, their quality of life and freedom would be positively impacted by digital tools that bring peace of mind with respect to their disease.

A particularly important area of focus will be nocturnal respiratory function. In particular, hypoventilation (shallow breathing) and difficulties in coughing due to muscle weakness are frequent in SMA, often leading to respiratory insufficiency and to pulmonary infections, respectively.

P100

Electrophysiological assessment of motor unit patterns of the median nerve in adolescents and adults with spinal muscular atrophy (SMA)

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SMA affects multiple parts of the motor unit. Electrophysiological techniques might offer insights into disease severity and motor unit reserve, and potentially serve as therapeutic biomarkers. We investigated motor unit patterns and their correlation with clinical factors in adolescents and adults encompassing a large part of the SMA spectrum, by using an electrophysiological protocol including compound muscle action potential (CMAP) scan and motor unit number estimation (MUNE).

We conducted a national, cross-sectional cohort study of Dutch adolescents and adults with genetically confirmed SMA types 1c-4 and in a healthy control group. None of the patients used *SMN*-augmenting therapies. We performed the CMAP scan on the median nerve, and derived $CMAP_{max}$, MUNE absolute mean motor unit (MU) size, and largest unit size (in mV), as well as the relative contributions of large and small MUs to the total pool (as % of $CMAP_{max}$). We explored associations of these CMAP scan markers with clinical characteristics, including SMA type, *SMN2* copy number, age, disease duration, motor function scores (HFMSE, RULM, and ATEND), and current motor ability (categorized as unsupported sitters, supported sitters, and walkers).

We included 104 SMA patients (median age 39, range 13-67) and 65 controls (median age 58, range 13-79). SMA types included 1c (n=7), 2a (n=29), 2b (n=17), 3a (n=32), and 3b/4 (n=19). Forty-seven percent were unsupported sitters, 33% supported sitters, and 20% walkers.
CMAP scan markers differed between patients and controls. In healthy controls, age had an inverse correlation with MUNE and positive correlation with the mean MU size (as % of $CMAP_{max}$).
Within SMA, age did not correlate with $CMAP_{max}$ in any type. Disease duration negatively correlated with $CMAP_{max}$ in patients with SMA type 3b/4. Both age and disease duration negatively correlated with MUNE and positively correlated with mean MU size (as % of $CMAP_{max}$) in patients with SMA type 1c. $CMAP_{max}$ and MUNE correlated with disease severity, as reflected by SMA type, *SMN2* copy number, motor function scores, and current motor ability. Absolute MU sizes (mean MU and largest unit size) did not correlate with disease severity. However, the relative contribution of small as well as large units differed with disease severity. In more severely affected patients, larger MUs constituted a higher percentage of the total pool, also reflected by a higher mean MU size (as % of $CMAP_{max}$).

This study explored motor unit patterns in a wide spectrum of patients with SMA and longstanding disease. We observed differences in electrophysiologic markers between disease severities, in particular motor unit quantity and the composition of large and small units and their relative contributions to the total pool. Although these findings do not conclusively establish a correlation with age or disease duration, they show promise as a biomarker in other patients than infants.

P101

Comprehensive digital health solution to improve the independence of people living with spinal muscular atrophy (SMA)

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Life with spinal muscular atrophy (SMA) is a constant balance between independence and everyday risks such as assistive-equipment failures, wheelchair accidents, and so forth. Seemingly harmless activities of daily living can quickly lead to emergencies (respiratory distress during sleep, for example) if people have no tool to help or to call for help.

In this poster, we present an ongoing study leveraging digital health technologies (non-invasive ambient sensors, wearable devices, and mobile apps) to make people with SMA safer and more independent.

The aim is to develop and test technologies to monitor some of the most significant risks of emergencies as well as more subtle health degradations, and to allow patients to react by calling for help and by sharing insights from key health measures and digital biomarkers with caregivers, to initiate prevention efforts.

As patients shared with us during pre-study interviews including caregivers, their quality of life and freedom would be positively impacted by digital tools that bring peace of mind with respect to their disease.

A particularly important area of focus will be nocturnal respiratory function. In particular, hypoventilation (shallow breathing) and difficulties in coughing due to muscle weakness are frequent in SMA, often leading to respiratory insufficiency and to pulmonary infections, respectively.

P102

SMA EFFORT: A New Approach to Perceived Physical Fatigability Assessment

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Fatigue is an intractable symptom of the SMA experience, evident through multidimensional patient-reported outcome measures. The most significant fatigue dimension in SMA is physical fatigability, which includes both performance-based and patient-reported measures. Perceived physical fatigability (*PPF*), defined as a whole-body measurement of one's susceptibility to fatigue with physical activity of fixed intensity and duration, has not been studied in SMA. Various multidimensional fatigue assessments used to characterize *PPF* in SMA have generated inconsistencies in assessment methodology and discordance between patient performance and patient reported physical fatigue-related experiences. These discrepancies have contributed to an inadequate understanding of the underpinnings of *PPF* in SMA. Using a previously established framework, we created an SMA-specific item bank to assess *PPF* (SMA EFFORT).

Participants (≥12 years; confirmed SMA) from four international registries completed a self-survey, consisting of 108 unique items organized across 33 distinct activities; all activities were relevant to the SMA phenotypic spectrum. Participants rated *PPF* from 0 = No Fatigability to 5 = Severe Fatigability, for activities that they can currently perform. SMA EFFORT (%) composite scoring was established to compare *PPF* within and across functional groups, irrespective of baseline function. Percent scores were calculated as the sum of item ratings divided by the number of items rated multiplied by 5. Descriptive statistics were collected, and one-way analysis of variance (ANOVA) was used to examine association with age, and compare differences by functional status, respiratory support, and disease-modifying therapy (DMT).

Among 118 respondents, 45% were sitters, 40% non-sitters, and 15% walkers, with an average age 41.3 years (range 14-78); 48% were male; and 61% were on DMT. SMA EFFORT score was not associated with age ($p = 0.18$). SMA EFFORT scores varied across functional groups ($p = 0.02$), notably between non-sitters ($\bar{x} = 31.9\%$, $SD = 19.4$) and sitters ($\bar{x} = 23.1\%$, $SD = 13.8$), ($p = 0.02$), and those with ($\bar{x} = 31.6\%$, $SD = 16.9$) and without respiratory support ($\bar{x} = 24.4\%$, $SD = 16.3$), ($p = 0.03$). Participants treated with DMT showed similar SMA EFFORT scores ($\bar{x} = 26.9\%$, $SD = 17.1$) to those without treatment ($\bar{x} = 26.3\%$, $SD = 16.4$), ($p = 0.84$).

Physical fatigability is a hallmark of the SMA patient experience. While evidence exists for contributing factors, their relative contribution to SMA-related physical fatigability remains unclear. The SMA EFFORT, contextualized by activity intensity and duration, provides a standardized framework for *PPF* ratings. Our tool aims to improve our comprehension of experienced physical fatigability to better gauge the impact of DMT on patient well-being. The SMA EFFORT will undergo refinement before validation to establish its psychometric properties.

P103

Exploring the impact of disease modifying therapies on quality of life and participation in adults with Spinal Muscular Atrophy – The lived experience

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Adults living in the United Kingdom (UK) with Spinal Muscular Atrophy (SMA) are prescribed Nusinersen and Risdiplam via a Managed Access Agreement. Little is known about the lived experience of treatment. Limited data from Europe suggest that adults living with SMA do not feel that the impact of treatment is adequately captured by the outcome measures currently used. The aim of this study is to explore the impact of treatment on the quality of life and participation of adults living with SMA in the UK.

A qualitative, phenomenological approach was used to explore the lived experience from the patient perspective. Data were collected via online focus groups and analysed using Thematic Analysis.

Seven participants aged 27 – 63 years old with type two and three SMA participated in four hour-long focus group interviews. Four have received Nusinersen and three Risdiplam for 1-3 years. Preliminary results suggest remaining independent and preventing functional deterioration are key motivators for treatment. Participants felt that treatment helped to reduce fatigue and contribute to improvements in mental health, leading to increased participation in daily activities. Improvements in respiratory function and bulbar symptoms were observed by participants with type 2 SMA receiving Risdiplam. Improved upper limb function in a cohort of participants has lead to increased independence in personal care, feeding and kitchen tasks. All participants felt the benefits outweighed any inconvenience associated with treatment. Further data analysis and theme development will be conducted.

Disease modifying therapies have a positive impact on quality of life and participation of adults living with SMA in the UK. Improvements in bulbar function and non-motor symptoms such as fatigue and mental health have been observed. These changes are important to participants but are not adequately captured by current outcome measures.

P105

A close look at motor function changes during nusinersen treatment in symptomatic children with SMA types 1,2 and 3

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The Hammersmith Functional Motor Scale-Expanded (HFMSE) is a validated outcome measure for monitoring changes in functional strength during the natural course of the disease and during drug treatment in patients with spinal muscular atrophy (SMA). The HFMSE consists of 33 items on a 3-point scale (0-2 points per item; range 0-66 points). HFMSE total scores are used to address therapeutic effectiveness, but it is unknown which items, and which item-score changes are most common in treated patients. Insight into potential patterns of muscle function improvement could help to manage treatment expectations and facilitate goal setting in physical therapy and rehabilitation. Therefore, the aim of this study was to analyse changes in HFMSE item-scores in children with SMA types 1c-3a treated with nusinersen over a period of six to twenty months.

We enrolled all patients with SMA in the Netherlands who were eligible for treatment between May 2017 and August 2019 and had the loading and 5th dose of nusinersen. We stratified the patients according to motor ability at start of treatment (non-sitters, sitters, and walkers) and evaluated all positive (score increase) and negative (score decrease) score changes (e.g. count and percentages) for each patient, and for each item. Frequency distribution of score changes at item level were analysed in each subgroup and in overall patients.

Fifty-four children were included (non-sitters n=11, sitters n=31, walkers n=12). Median age at baseline was 5.1 years (range 1.5-9.8), with a mean interval between measurements of 15.2 months (range 6.9-20.1). The majority of the children (83%) improved in item score in at least 1 item. Non-sitters showed a limited response, with only 36% improving in score in 1 or more items. Most Sitters (94%) and all walkers improved in 1 or more items. Most achieved score changes were changes from 0 to 1 (*unable* to *performs with compensation*). The items 'sitting' for non-sitters, items containing rolling for sitters and items containing high kneeling for walkers demonstrated highest percentages of improvement. The items assessing hip flexion showed the largest decrease (6-7%).

Motor function change on the HMFSE in symptomatic children with SMA type 1-3 during nusinersen treatment, is characterized by the acquisition of new motor skills with compensation strategies. Improvement on HMFSE-items is dependent on motor ability at start of treatment. Not all items show equal responsiveness. Non-sitters showed limited response, sitters mostly improved on items that assess rolling, and the majority of walkers improved on items that assess half-kneeling. The ability of hip flexion in supine position was most frequently lost.

The presented data can be used in physical therapy to determine treatment goals and for expectation management. It encourages to look beyond HFMSE total scores to interpret treatment effects in children with SMA.

P106

Protocol for a Delphi consensus panel on assessing clinically meaningful treatment outcomes in adults living with SMA

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Adults living with spinal muscular atrophy (SMA) are a heterogeneous population, with a wide range of functional abilities, severity of symptoms and complications. These complexities, compounded by limitations of the relevant clinical outcome assessments to represent this wide range of function, create challenges in measuring response to therapeutic intervention. Currently, pharmacological access criteria and scientific research place a disproportionate weight on gross motor function assessments, whilst gaps exist in other meaningful aspects of fine motor function, respiratory function, bulbar function, and fatigue. Current assessment of treatment response and observations of improvement are not well measured for adults living with SMA. Important questions about when a treatment is effective and how to assess treatment efficacy remain unanswered, which can negatively impact patient access to and quality of care. This study specifically aims to derive consensus on clinically meaningful treatment outcomes and constructs in adults living with SMA.

A modified Delphi study will be conducted, consisting of two online survey rounds and a final consensus meeting. A steering committee of six medical experts and three patient advocates will provide scientific oversight throughout the study. Panellists will be double blinded to the study sponsor, study investigators and other participants for the entirety of the study. Eligible participants will include 25 neurologists, 25 allied health professionals and six patient advocacy group representatives from the USA, UK, France, Germany, Italy and Spain. The online survey will be conducted in two rounds; the first round will be explorative and aims to capture key concepts of importance. The second round will be developed based on the results from the first round and will aim to understand areas that are nearing consensus and identify any points that require clarification or further exploration in the final consensus meeting. Across both survey rounds, panellists will be asked to provide detailed insights on the key outcomes that should be assessed to determine what constitutes a clinically meaningful treatment outcome in adults living with SMA. Panellists will then participate in a consensus meeting, to discuss and consolidate their responses and reach their final conclusions. Consensus will be defined as ≥80% of panellists showing disagreement or agreement. This study aims to determine expert consensus on the aspects of SMA that should be considered when determining meaningful treatment outcomes in adults living with SMA, and how such outcomes should be assessed and interpreted. Throughout the study, definitions of a meaningful treatment response will also be explored. Outputs from the study will be used to educate various stakeholders on the true value of defining and utilising meaningful treatment outcomes individualised to adults living with SMA, helping to support improvements in and access to adult SMA care.

P107

Post hoc analysis of the SMA Independence Scale-Upper Limb Module (SMAIS-ULM) in individuals with Type 2 and non-ambulant Type 3 SMA using SUNFISH Part 2 data

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Individuals with Type 2 and non-ambulant Type 3 spinal muscular atrophy (SMA) experience proximal muscle weakness, which can contribute to difficulties in carrying out activities of daily living (ADLs) independently. The level of independence of individuals with SMA can be heterogeneous and dependent on age, as well as other contextual factors. The SMA Independence Scale-Upper Limb Module (SMAIS-ULM) is a validated clinical outcome assessment, designed to assess the level of support required for ADLs related to upper limb function in individuals with Type 2 and non-ambulant Type 3 SMA. The SMAIS-ULM patient-reported and caregiver-reported versions were assessed in SUNFISH (NCT02908685), a multicentre, two-part, randomised (2:1, risdiplam: placebo), placebo-controlled, double-blind study in a broad population of patients with Type 2/3 SMA (2–25 years at enrolment). This research presents a post hoc analysis of SMAIS-ULM, which aimed to explore the impact of risdiplam on upper limb function, and levels of independence in children, teenagers and adults with SMA.

All analyses were conducted on SMAIS-ULM patient-reported (n=68 patients; ≥12–25 years) and caregiver-reported (n=180 caregivers of patients 2–25 years) data from SUNFISH Part 2 at Month 12 (placebo-controlled period). Mixed Models for Repeated Measures analyses examined mean change in SMAIS-ULM total score from baseline to Month 12, by treatment arm and age subgroup (2–5 years; 6–11 years; 12–17 years; 18–25 years). Responder analyses were used to establish the proportion of patients who showed improvement or stabilisation (≥0) compared with any decline (<0), in SMAIS-ULM total score from baseline to Month 12.

A numerical increase from baseline in patient-reported (≥12–25 years) SMAIS-ULM mean total scores was observed in the risdiplam age subgroups, compared with a decline in the scores of the placebo age subgroups at Month 12. SMAIS-ULM reported by caregivers (of patients 2–25 years) similarly showed numerical increase in favour of risdiplam across age subgroups, with the exception of the 18–25 years subgroup. A larger proportion of individuals in the risdiplam group showed improvement or stabilisation in SMAIS-ULM total score from baseline, compared with placebo. This finding was observed across all age subgroups, for both patient-reported and caregiver-reported SMAIS-ULM total scores.

The ability to carry out ADLs independently is a key determinant of health-related quality of life in individuals with SMA. Current findings highlight the benefit of risdiplam on upper limb function, and levels of independence in ADLs, across children, teenagers, and adults with more advanced disease. Patients on risdiplam were more likely to maintain or gain independence in ADLs, and less likely to lose independence than those on placebo. The desire to maximise or maintain independence is important to individuals with SMA, their families and caregivers.

P108

Bulbar function outcomes in children with SMA type-1 treated with onasemnogene abeparvovec

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In the natural history of Spinal muscular atrophy type 1 (SMA1) bulbar dysfunction is a well-known aspect of the disease, with more that 50% of infants requiring feeding support by 8 months of age.[1] Difficulties reported include jaw dysfunction, mastication fatigue, aspiration and intelligibility problems.[2] With the availability of onasemnogene abeparvovec (OA) gene therapy it is it vital to assess and monitor bulbar function outcomes and characterise how bulbar dysfunction responds to treatment.

A retrospective service evaluation using case note review to analyse early bulbar outcome data in 25 children with SMA1 treated with OA at Great Ormond Street Hospital. Bulbar function was assessed using the Children's Eating and Drinking Ability Scale (CEDAS, previously named paediatric functional oral intake scale (PFOIS)) at baseline (pre-treatment), then 6, 12, 18 and 24 months post-treatment. Patients are scored from 1-6, with a score of 1 representing severe bulbar dysfunction; patients are nil by mouth, requiring tube use for all nutrition and hydration gradually increasing to a maximum score of 6 with age-appropriate food and drink intake with no restrictions. [3]

25 children were treated between 1 month - 88 months (7.5 years) of age, median age was 6 months. All patients had baseline and 6 months CEDAS/PFOIS scores. The median CEDAS/PFOIS score at baseline was; 6 (range 1-6, n=25) at 6 months; 6 (range 1-6, n=25), at 12m; 6 (range 1-6, n=14), at 18m; 6 (range 1-6, n=7) and at 24m; 6 (range 1-6, n=7).
Among the 16 patients treated under 1 year of age, median baseline CEDAS/PFOIS score was 6 (range 1-6, n=16) and the median score remained at 6, at 6 months (range 2-6, n=16) and at 12 months (range 3-6, n=11).
12 patients were treatment-naïve, 13 patients had previously received disease-modifying therapies (DMT), 12 with nusinersen and 1 risdiplam. Of the treatment-naïve, median age was 4 months (range 1-17m), the median CEDAS/PFOIS score at baseline was 6 (range 1-6, n=12). Of those with previous DMT median age was 18 months (range 3-88m), the median CEDAS/PFOIS score at baseline was 5 (range 1-6, n=13).

Our results show that children treated with OA maintain bulbar function, with scores stable across the 24 month-follow up. Patients treated under 1 year of age showed not only stability of bulbar function, but some also improved over time. These findings indicate the need to further investigate the effects of various disease modifying treatments to preserve bulbar function in both pre-symptomatic and symptomatic patients.

P109

Real-World Outcomes of Infants who Initiate Risdiplam Under 2 Months of Age for the Treatment of Spinal Muscular Atrophy (SMA) in the US

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Risdiplam (EVRYSDI®) is a once-daily, orally administered, survival of motor neuron 2 (*SMN2*) pre-mRNA splicing modifier approved by the US Food and Drug Administration (FDA) to treat pediatric and adult patients with SMA. Early diagnosis of SMA has increased due to prenatal and newborn screening and treatment should be administered as early as possible to preserve motor neurons. In May 2022, the FDA approved a label extension for risdiplam to treat infants under 2 months of age based on data from the RAINBOWFISH clinical study (NCT03779334). Analysis of data as they become available will help characterize the real-world experience of risdiplam in infants. Here we describe a planned study design.

This will be a multicenter, retrospective chart review in the US. The objective of the study is to characterize the real-world experience of infants with SMA under 2 months of age treated with risdiplam. Clinical sites with relevant experience will be included in the study. Data to be collected will include baseline demographics (e.g. age, sex, race/ethnicity) and disease characteristics (e.g. diagnosis method, *SMN2* copy number, SMA type, feeding and ventilation status), when available. Information on risdiplam treatment (e.g. dose of risdiplam administered, age at first dose, time from diagnosis to treatment), other treatments administered, and safety and effectiveness outcomes will also be captured, when available.

A better understanding of the real-world use and experience with risdiplam in the newborn population will complement the clinical efficacy and safety data collected from the RAINBOWFISH study.

P110

A retrospective, non-interventional cohort study to compare adults with SMA receiving risidiplam with untreated patients: Study design

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Risdiplam (EVRYSDI®) is a disease-modifying therapy (DMT) approved for the treatment of spinal muscular atrophy (SMA). The efficacy of risdiplam has been demonstrated in clinical trials that included adult patients, but the real-world effectiveness of risdiplam in adults has not yet been extensively investigated. Natural history studies have shown a slow, progressive decline in motor function in adult patients with SMA. It remains unclear how risdiplam may influence this trajectory in a real-world clinical setting.

This non-interventional, retrospective cohort study aims to describe adult patients with SMA in terms of their demographic and clinical characteristics, treatment patterns and healthcare resource utilisation. If possible, it will also explore how adult patients with SMA newly treated with risdiplam compare with patients who are not receiving DMTs. This study will use data from seven registries (clinician and patient reported) from within the TREAT-NMD network (Australia, Belgium, Canada, Czech Republic & Slovakia, Germany & Austria, Ukraine, and UK & Ireland) to identify adult patients with SMA treated with risdiplam and compare with an untreated patient population, in registries deemed feasible for a comparative analysis. Appropriate adjustment methods will be explored to adjust for potential confounders, and appropriate statistical models will be used to perform comparisons with untreated patients.

The analysis of this study is ongoing as of the submission of this abstract. Available data from the comparative analyses will be presented.

There is limited published real-world evidence of risdiplam in adult patients with SMA. Further research is needed to better understand the effectiveness of risdiplam in this understudied population and to compare treated and untreated patients. This study will provide additional evidence to fill this gap and further support the understanding of treatment effectiveness in adults with SMA.

P111

Assessing the feasibility of TREAT-NMD registries for characterising adult patients with SMA receiving risdiplam treatment

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Risdiplam (EVRYSDI®) is a disease-modifying therapy (DMT) approved for the treatment of spinal muscular atrophy (SMA). The efficacy of risdiplam has been demonstrated in clinical trials that included adult patients but the real-world effectiveness of risdiplam in adults has not yet been extensively investigated. Aetion, Roche and TREAT-NMD are collaborating in a real-world registry-based study to understand the effectiveness of risdiplam in adults by comparing outcomes of adult patients treated with risdiplam with those of adult patients not receiving DMTs.

A fit-for-purpose (FFP) assessment was conducted to determine the feasibility of the SMA registries within the TREAT-NMD network to provide data for pre-specified elements. The FFP assessment consisted of a pre-feasibility and a feasibility assessment.

Based on risdiplam availability in adults, 10 SMA registries within the global TREAT-NMD network were contacted for the pre-feasibility assessment: seven registries were based in Europe (Belgium, Spain, UK & Ireland, Czech Republic & Slovakia, Ukraine, Germany & Austria, and Sweden), two in the Pacific region (Australia and New Zealand) and one in North America (Canada).
In the pre-feasibility stage, four registries were excluded because of a low number of adult patients with SMA, a limited number of patients receiving risdiplam or due to data availability issues. Subsequently, seven registries, (Australia, Belgium, Canada, Czech Republic & Slovakia, Germany & Austria, Ukraine, and UK & Ireland) went through the feasibility assessment.
Data availability and completeness were examined across the seven registries included in the feasibility assessment. Around three quarters (78%) of adult patients had a genetic diagnosis of SMA and 82% had a record for DMT. Patient-reported outcomes and motor function measures were limited and heterogeneously reported across registries. Based on overall rankings, registries were categorised into three tiers, ranging from the most to least FFP for inclusion in this specific study. Registries from Belgium and Czech Republic & Slovakia were in the highest tier as they met most data element requirements with a high level of completeness. Registries from Australia, Canada, and Germany & Austria were in the middle tier as some data were not collected systematically for the purposes of this study. Registries from UK & Ireland and Ukraine were in the lowest tier due to missing data or certain data elements not being collected in the context of the study objectives. All registries included in the highest and middle tiers had aspects of clinician verification, whilst others were patient reported.

P112

Biomechanical gait assessment of spinal muscular atrophy type 2 and 3: Insights from an ongoing study

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Spinal Muscular Atrophy (SMA) is a leading genetic factor in infant mortality, characterized by progressive motor neuron loss and generalized muscle weakness. Recent therapeutic advancements, including Nusinersen and Zolgensma, have revolutioned SMA management, offering hope for enhanced patient outcomes. However, comprehensive insights into the impact of these treatments on motor function, particularly gait, remain limited. Understanding the characteristics of normal and pathological gait can aid physiotherapists and healthcare professionals in adapting strategies to reduce energy consumption and enhance functionality and independence in locomotion. NedAMHPlus/IBV, a software application developed by the Biomechanics Institute of Valencia (IBV), utilizes data from dynamometric platforms, photogrammetry systems, and surface electromyography to assess the functional capacity and repeatability of human gait. The primary objective of this research project is to evaluate the progression of gait in pediatric patients with SMA type 2 and 3 receiving pharmacological and physiotherapeutic treatment.

This doctoral research project focuses on evaluating the motor function and gait patterns of pediatric SMA patients type 2 and 3 undergoing pharmacological and physiotherapy interventions. Through dynamic, kinetic, and electromyographic assessments, differences in gait patterns between SMA patients and typically developing children are sought to be discerned. The research utilizes an observational case-control design, approved by the ethics committee of the University and Polytechnic la Fe Hospital, comparing biomechanical data from SMA cases with controls from the general population. For both groups, we will identify children aged 5 to 17 with a minimum ambulatory capacity of 25 meters, without orthoses or assistive devices, and possessing the cognitive ability to follow simple assessment instructions. Additionally, the case group must exhibit characteristic symptoms of each subtype of SMA and be part of the national registry of SMA patients (FUNDAME). Exclusion criteria will also be considered. Measurement sessions will be held at the IBV facilities. The independent samples t-test will be utilized while verifying the following assumptions: normality using the Shapiro-Wilk or Kolmogorov-Smirnov test, homoscedasticity through the Levene's test, and the assumption of independence.

In this ongoing study, we anticipate observing significant differences in both dynamic and kinematic aspects when analyzing gait patterns between individuals with SMA type 2 and 3 and those with typical development.

The findings of this study have substantial potential to enhance treatment strategies, thereby improving the quality of life and mobility of SMA patients. Bridging the knowledge gap in gait patterns in SMA significantly contributes to better therapeutic interventions for affected children.

P113

Bone density and muscle mass in nusinersen-treated SMA individuals

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Bone mineral density (BMD) is lower in individuals with spinal muscular atrophy (SMA) compared to those with other neuromuscular disorders and healthy peers. Low BMD increases risk for fractures and other co-morbidities. Multiple factors contribute to low BMD, including muscle weakness and decreased weight bearing. A strong relationship between lean body mass and BMD has been established in healthy populations. The purpose of this study is to determine the relationship between muscle mass and BMD in SMA individuals treated with nusinersen.

Data were collected from 2018-2021. BMD and fat-free mass were assessed in ambulatory individuals with SMA using dual-energy x-ray absorptiometry (DEXA). BMD z-score was used during analysis as an additional comparison across individuals. Fat-free mass index [FFMI =fat-free mass (grams) / height^2 (m^2)] was used to normalize fat-free mass. Strength of knee flexion (KFLEX) and knee extension (KEXT) were measured using hand-held dynamometry. Regression analysis was performed to determine the associations of FFMI and duration of nusinersen treatment with BMD. Paired samples t-tests were used to compare strength and BMD z-score between visits. Wilcoxon signed-rank tests were used for KEXT and BMD since they were not normally distributed.

Thirteen ambulatory SMA individuals, mean age 31.2 years (range 12.7-56.9) and mean BMI 24.8 (range 15.4-38.1) were followed for 2 visits separated on average by 7.9 months (range 5.0-20.6). At visit 1, FFMI was not significantly associated with BMD z-score (r =.403; p=.172). At visit 2, FFMI was significantly associated with BMD z-score (r=.625; p=.02). Nine individuals (69.2%) were treated with nusinersen for a mean duration of 2.2 years (range=1.1-6.4). When nusinersen treatment duration was included in the model, the relationship was even stronger at the second visit (r=.773; p=.03). BMI was stable between visits with an average change in BMI of -.03 (p=.88). Mean changes in FFMI (Δ=-.04, SD=.13; p=.008) and KFLEX (Δ=2.2, SD=2.8; p<.001) between visits were significant. The changes in BMD (Δ=-.03, SD=.06; p=.722), KEXT (Δ=1.30, SD=.35; p=.161), and BMD z-score (Δ=-.33, SD=.94, p=.316), were not significant.

Low BMD is a known co-morbidity in SMA with several possible causes. Disuse atrophy, osteoporosis and low vitamin D levels associated with fractures are reported mostly in non-ambulatory SMA types. We found an association between fat-free mass and BMD in ambulatory SMA, which was even stronger in nusinersen treated individuals. Knee flexion strength improved in treated patients, without a similar change in BMD. Longitudinal follow-up with more sensitive imaging tools will be helpful to further understand muscle composition and BMD.

P114

Spine MRI as potential biomarker of disease progression in SMA patients treated with Nusinersen: A pilot study

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Nusinersen is a recently approved treatment for spinal muscular atrophy (SMA) that appears to prevent the degeneration of alpha motor neurons (αMN) in the spinal cord (SC), slowing the progression of the disease[1]. Quantitative magnetic resonance imaging (MRI) of the cervical spine has been widely demonstrated to detect and quantify spinal cord atrophy, particularly gray matter atrophy, resulting from motor neuron and white matter degeneration[2,3,4].
The aim of our study is to introduce new potential MRI biomarkers to evaluate the efficacy of Nurinsen treatment in halting or delaying motor neuron degeneration, focusing on differences between adults treated at advanced stages of the disease and infants treated at earlier stages.

Three adult and five infant SMA patients under treatment with Nusinersen underwent longitudinal clinical (HFMSE)[6] and MRI examinations from baseline to 36-month follow-up, with 6 timepoints (T0, T8, T12, T24, T36 months). The MRI protocol included a 3D T2w and T2*GRE sequence centered on the cervical spine. Total spinal cord area (tCSA) and gray matter area (gmCSA) were automatically segmented at the C3-C4 level using STC (Spinal Cord Toolbox)[5]. We then performed a longitudinal assessment of clinical and imaging outcomes in SMA patients, comparing the progression tCSA and gmCSA over time between adults and infants who received earlier treatment.

Patients' motor function remained relatively stable in adult patients, whereas an improvement in motor performance has been detected in the pediatric cohort.
Regarding the MRI evaluation of the SC, all adult patients showed a slight, non-significant increase in total cross-sectional area (tCSA) at TP36 compared to baseline (meanCSA=+0.423mm^2; p=n.s.). Two of the three adult patients showed a slight decrease in gray matter cross-sectional area (gmCSA) at TP36, whereas one patient exhibited a subtle increase in gmCSA, but without reaching statistical significance in either cases, indicating substantial stability.
In the pediatric population, global tCSA showed a slight, not significant increase (ΔCSA=+1.150mm2; p=n.s.) at TP36 compared to baseline. GmCSA statistically significantly increased in all patients at the last timepoint (meanCSA=+0.42mm^2; p=n.s). It is worth noting that during childhood and adolescence, the spinal cord and subsequent tCSA and gmCSA increase due to the physiological growth of the spinal cord. Therefore, at this stage of the study, we can only hypothesize a potential beneficial effect of Nurinsen in preventing motor neuron degeneration in the cord gray matter.

In this study, SC qMRI suggests a higher response to Nurinsen in infants who received therapy significantly earlier than adults, with greater improvement in motor function and apparently regular spinal cord growth, reducing total spinal cord and gray matter atrophy. Further studies on larger cohort are necessary in order to confirm these findings.

P115

Evaluation Of Motor Outcome in Patients with Spinal Muscular atrophy (SMA) treated with Risdiplam in Georgian cohort

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Spinal muscular atrophy is a rare genetic, progressive neurodegenerative disease, causing progressive muscular, respiratory, and bulbar weakness. The treatment for SMA was primarily supportive until disease modifying therapy was approved by FDA, which showed dramatic improvements in motor milestones. Risdiplam is an oral SMN2 enhancer, approved for use in children 2 months of age and older. Interdisciplinary approach is essential to treat patients with SMA. In 2022 the medication Risdiplam was registered in Georgia and became available for all patients.
In present study, we observed the relationship between new medical treatment and functional motor outcome.

The observational study includes 42 patients from 0-18 years. Non of them was treated before 2022. They were assessed before and 12 months after the treatment. We evaluated the motor function of this SMA patients with validated motor assessment tools: Revised Hammersmith Scale (RHS), Infant test of neuromuscular disorders (CHOP-INTEND) to perform different qualitative motor function. Risdiplam in standard doses was administered orally, once per day. 30 patients underwent interdisciplinary rehabilitation therapy. We investigated changes in motor function scores during treatment correlated with SMA types.

In our cohort 42 patients are under observation. 8 patients are full-time and 1-on part-time mechanical ventilation.
SMA type1: 6 patients were<12-month-old. Three of them on ventilation, with tracheostomy. Before treatment, all patients underwent a CHOP-INTEND motor scores ranged between 3-32 (average score 10). The patients treated earlier (<12m) archived larger improvements in motor function. After 1 year of treatment two of them removed from ventilation and scores increased 7–54 (average score 31).
SMA type 2: 19 patients (2-18y) RHS scores before treatment were 0-20 (average score 4.8), after 12 months of treatment RHS - 0-24 (average score 8.4). In 3 patients scores did not improve.
SMA type 3: 10 patients (3-18y) RHS scores before treatment were 6-51 (average score 34), after 12 months of treatment - 12-61 (average score 43.4).
We found correlation between the age at treatment initiation and motor improvement measured by the change in motor function.
In patients (6 SMA type 1 and 1 SMA type 2) who have been on ventilation continuously, the treatment didn't show any changes in motor function.
Thirty patients who received combination of medical and interdisciplinary rehabilitation therapy had better outcomes. The scores before treatment were 0-51 (average score -15.9). after treatment 3-61 (average score 25.3).

In our study no patients died, and side effects were mild and transient. Our data confirms that early initiation of treatment positively affects SMA type 1. Type 2, and type 3 also gained or stabilized motor function scores. Combination of Risdiplam and rehabilitation therapy is effective in all types of children with SMA.

P116

Risdiplam in type 2 and 3 Spinal Muscular Atrophy: Results of a cohort of adult Italian patients

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To retrospectively investigate safety and efficacy of Risdiplam in a small cohort of Italian adult type 2 and non-ambulant type 3 Spinal Muscular Atrophy (SMA) patients.

Inclusion criteria were: clinical and molecular diagnosis of SMA2 or SMA3; treatment with Risdiplam and good therapeutic compliance; clinical data available at the first evaluation (T0 – beginning of administration) and at the last evaluation carried out after a period of at least 12 months (range 12-24 months). The Hammersmith Functional Rating Scale Expanded (HFMSE), the Revised Upper Limb Module (RULM) and the 32-item Motor Function Measure (MFM32) rating scales were administered.

We included 6 patients (5 SMA2 and 1 SMA3) with median age at first administration of 40 years (range 22-55) and an SMN2 copy number of 3 (4 patients) or 4 (2 patients). HFMSE in patient with SMA3 increased from baseline to last evaluation (+3 points). RULM in SMA3 also improved between T0 and last evaluation (+3 points). Conversely, patients with SMA2 had no significant changes, hence a steady state, of median HFMSE and RULM between T0 and the following time points (median change of 0 and +0.6 points respectively), although an improvement of MFM32 was observed (median change +5.2 points). Furthermore, all patients report an at least subjective benefit especially with bulbar functions including respiratory function, tone of voice (reduction of rhinolalia) and head control. No adverse reactions or drug intolerance were recorded in any patient.

Our data provide further evidence of Risdiplam safety and efficacy in adult SMA2 and SMA3, despite the size limitations of this sample. In particular, HFMSE and RULM showed an increase in scores for the SMA3 patient while in SMA2, despite the reported clinical improvements, the scores appear stationary. Therefore, in SMA2, among the currently used scales, MFM32 seems to be the most sensitive in detecting improvement in these patients. However, further tools seem to be needful to investigate the efficacy of the drug regarding bulbar functions.

P117

Assessing risdiplam utilization, adherence, and associated healthcare costs in patients with spinal muscular atrophy: Analysis of a US retrospective claims database

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Risdiplam is an oral medication recently approved by the US Food and Drug Administration (FDA) for the treatment of pediatric and adult patients with spinal muscular atrophy (SMA). Recommended dosing schedules require daily, presumably lifelong, use. To date, no studies have assessed risdiplam utilization, adherence, and its associated healthcare costs in a real-world setting.

We conducted a retrospective claims database analysis from January 1, 2020, to June 30, 2022. Patients with SMA who received risdiplam on or after August 7, 2020 (FDA approval date), and who had continuous enrollment for ≥3 months before and ≥6 months after risdiplam initiation date (index date) were included. Eligible patients were followed until the end of continuous enrollment, the end of data availability, or 1 year from index date, whichever came first. Percentage of days covered (PDC) for risdiplam was estimated and adherence was defined as PDC ≥80%. Patient characteristics and healthcare costs were summarized descriptively for patients who were adherent vs. non-adherent to risdiplam by SMA type and age group.

Eighty-six patients met study criteria: one SMA type 1 (1-year-old boy), 18 type 2 (mean±SD age, 7.9±5.7 years; 61% female), 47 type 3 (17.3±10.2 y, 55% f), and 20 with type 4 (38.2±11.6 y, 55% f). Within the data period, 43 patients (50%) were treated with risdiplam alone and 42 (48.8%) received nusinersen prior to risdiplam; nine patients switched back to nusinersen. One patient used risdiplam after nusinersen and onasemnogene abeparvovec. Within 1 year of risdiplam initiation, 18 patients (21%) discontinued risdiplam: three (17%) SMA type 2; eight (44%) type 3, and seven (39%) type 4. Mean PDC was 89% for the overall cohort, ranging from 88% for SMA type 4 to 97% for type 1. Overall, 83.7% of the patients were adherent to risdiplam, ranging from 75.0% for SMA type 4 to 100% for type 1. Percentage of patients adherent to risdiplam was 76.5% for age 6–12 years (n=17), 80% for 12–17 y (n=15), 85% for ≥18 y (n=41), 90% for 3–5 y (n=10), and 100% for 0–2 y (n=3). Non-adherent patients incurred greater total healthcare costs across SMA types 2–4. Median all-cause healthcare costs for the two cohorts were $21,475.4 vs. $356,524.6 in SMA type 2; $14,909.1 vs $56,113.0 in type 3; and $4,594.1 vs. $16,817.0 in type 4. For adherent patients, those with PDC between 90–100% had less all-cause healthcare costs vs. those with PDC between 80–90%.

Non-adherence to SMA disease-modifying therapies could have important clinical implications and cost impacts. In our overall cohort, approximately one in six SMA patients was non-adherent to risdiplam, with a greater rate (25%) observed for patients with SMA type 4. Patients aged 6–12 years had the lowest adherence rate. Non-adherence to risdiplam was associated with greater total healthcare costs. Discontinuation of risdiplam within the first year of treatment was nontrivial.

P118

Oro facial strength in symptomatic type 1 SMA patients treated with nusinersen: Results from a prospective study involving 4 centers Milano - Rome - Brussels -Ghent

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Spinal muscular atrophy (SMA), a genetic neuromuscular disease caused by the lack of survival of motor neuron (SMN) protein, is characterized by muscular atrophy and respiratory and bulbar dysfunction, in particular in type 1 SMA. Swallowing function remains poorly studied, mainly because investigation tools such as Fiberoptic Endoscopic Evaluation of Swallowing (FEES) and Videofluoroscopic swallowing study (VFSS) are often not available or poorly tolerated by young children. A user friendly IOPI system measurement was recently developed to objectively evaluate orofacial strength mainly in type 2 and 3 SMA patients.
The main objective of this study is to investigate the level of impairment of muscles involved in the oral phase of swallowing (lips and tongue) in a cohort of type 1 SMA children treated with nusinersen when compared to healthy controls. Secondly, we aim to correlate lip and tongue strength with known key predictors of phenotype severity (CHOP Intend, age at first symptoms, SMN2 copy number), and with indicators of feeding-nutritional status such as the need for gastrostomy, the body mass index (BMI).

By combining the results of 4 independent centers that were actively investigating IOPI measurement in the context of a prospective study, we recruited 20 patients with a confirmed genetic diagnosis of symptomatic type 1 SMA. All patients were treated with nusinersen for a minimum of time 2,5 years and all had at least one IOPI measurement at the anatomical level of the tongue and lips at a median age of 5,4 years. The IOPI data were compared against age and gender-matched controls and published normative data where available.

Preliminary results showed that the oral pressure is clearly lower in both anatomical localizations (lip: n=12/20 patients – tongue: n=15/20 patients) when compared to typically developing children (lip – $p<0.01$ - tongue: $p<0.001$). There is a positive correlation between CHOP Intend score and muscle (lip) pressure ($r=0.723$, $p=0.012$). There was no difference between the median lip pressure between those without and with gastrostomy: (7 vs 6 kPa), whereas the median tongue pressure was higher in those not requiring gastrostomy (7.5 vs 4 kPa). Other correlations with key indicators of disease severity and feeding nutritional status are ongoing.

While patients treated with nusinersen clearly achieve prolonged survival and clear general motor progress, IOPI measurement could be performed in clearly objectify the weakness of oro facial musculature in this population.

P119

Evaluating sleep disordered breathing in pediatric patients with neuromuscular disorders: Insights from a retrospective cohort study

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Sleep-disordered breathing (SDB) is a common concern in pediatric patients with neuromuscular disorders (NMD) due to respiratory muscle weakness and declining lung function. The current practice guideline recommends polysomnography as the preferred option for assessing the need for noninvasive ventilation (NIV) in symptomatic NMD patients when pulmonary function tests (PFT) and overnight oximetry (ONO) show normal results. However, there is low certainty of evidence supporting this recommendation, highlighting the need for careful evaluation of individual patient needs and circumstances. Our objective was to compare the efficacy of spirometry and polygraphy with transcutaneous capnography (PG+trCO2) in detecting SDB and assess PG+trCO2's role in diagnosing SDB in patients with DMD and SMA.

A retrospective study at Children's Clinical University Hospital, Riga, Latvia involved evaluating the PG+trCO2 and spirometry data of DMD and SMA diagnosed patients.

70 patients were included; 37% had PG+trCO2, 64% spirometry, and 20% both. 77% of those with PG+trCO2 showed alterations such as sleep tachypnea and hypoventilation. Of the 45 who had spirometry, 47% demonstrated alterations in their FEV1 and FVC, ranging from mild to severe restrictive breathing. Of the 14 patients who had both evaluations (PG+trCO2 and spirometry), 21% had alterations only in PG+trCO2. This resulted in a change in their treatment regimen, with the addition of NiV.

Polygraphy and transcutaneous capnography, when combined with spirometry, offer enhanced detection of SDB compared to using spirometry alone. The study underscores the importance of incorporating PG+trCO2 into routine diagnostics for early SDB detection in pediatric NMD patients. A proposed "PG+trCO2 for All" policy suggests annual screenings for NMD patients, but further research is imperative to determine optimal monitoring strategies.

P120

Patients' perceptions of the effects of Spinraza® according to their status as a responder or non-responder

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In clinical trials and daily practice, patients with spinal muscular atrophy (SMA) treated with a disease-modifying therapy (DMT) are considered as responders (R) or non-responders (NR) based on the level of achievement in assessments with validated functional scales. In some countries, the "NR" designation may restrict further access to DMT. Based on our clinical experience, we hypothesized that small functional improvements or even stabilization that could have a positive impact on a patient's daily life might not be detected by the functional scales. The objective of this international multicenter study is to compare patients' conditions captured by functional scales (retrospective part) with patients' perceptions of treatment efficacy (prospective part) after 12 months of Spinraza® stable-dose treatment, as well as to individualize aspects of patient perception that are not measured by the current functional scales. Based on the literature, an order of priority was established for the functional scales according to the type of SMA and the age at treatment-initiation (i.e. patients with SMA II from 24 months onwards: 1) HFMSE 2) MFM 3) RULM). Patients were divided into three groups (Non-responder (NR), Responder non-clinically significant (RNCS) and Responder clinically significant (RCS)) whether they showed clinically significance improvement (ie, ≥4 points on CHOP Intend, ≥3 points on MFM32 and HFMSE) 15 months after starting treatment. For the prospective part of this study, we designed a quality-of-life questionnaire composed of 22 questions targeting important aspects and tasks of daily life for SMA patients (including balance, fatigability, swallowing, respiratory support) not captured by functional scales. A scoring system based on the Clinical Global Impression-Improvement scale, which uses 7 scores ranging from very much improved to very much worsened allowed patients to estimate whether a change was perceived after one year of stable Spinraza® dosing. This quantification of patients' impressions of RCS, RNCS and NR will be presented.
In total, 101 patients have been interviewed. 41 were classified as RCS, 18 as RNCS and 42 as NR. Among them, eigthy-eight feel that their general condition has improved after treatment.
Our results demonstrate that patients considered as non-responders based on motor function scales see stabilizations and/or improvements in various domains not considered by these scales. There were only very few differences between Responders and Non-Responders based on the quality-of-life assessment. While functional assessments are used in some systems to justify access to treatment or not, it might be important to consider their results with caution at the individual level.

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A combined examination of novel rapid bedside plasma-SMN analysis and muscle ultrasound may help to early screen & monitor children with spinal muscular atrophy in clinical setting

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Spinal muscular atrophy (SMA) is a genetic neuromuscular disorder characterized by progressive muscle weakness. In developing countries, delayed management is a common issue due to the difficulty in accessing genetic testing. Studies have reported the usage of muscle ultrasound (USG) and Survival Motor Neuron (SMN) protein measurement in the management of SMA patients. However, the optimal clinical utility remains unclear. This study aimed to optimize the usage of plasma-SMN analyses *and* muscle USG as means for SMA patients' screening and monitoring.

A combined study of a cross-sectional design followed by a 6-month prospective cohort design involving 49 SMA-genetically confirmed patients was performed. The plasma-SMN levels were measured using a novel in-house designed rapid SMN-meter and validated by immunoblotting using anti-SMN protein antibody and anti-alpha tubulin antibody as the references. Background scatter (echo intensity) analysis (BSC), muscle thickness, and architecture were used as USG parameters. Clinical scores were measured using standardized Hammersmith Functional Motor Scale- Expanded (HFMSE) and The Motor Function Measurement (MFM) score. Sensitivity, specificity, parameter changes, and correlation were analysed in the initial diagnosis and then calculated every 3 months.

The plasma-SMN measured using rapid SMN-meter showed high agreement with the immunoblotting result (kappa=0.82). Muscle USG which was used together with clinical score and plasma-SMN analysis had sensitivity of 93% (95%CI:90-96%) and specificity of 91%(95%CI:90-92%) in SMA screening. A *moderate* correlation (r=0.78) was noted between BSC and SMN level serial measurement with clinical scores during 6 months of clinical monitoring.

Using Muscle USG alone as screening tool may pose challenge. However, together with bedside rapid plasma-SMN measurement, it can become an efficient and non-invasive method for screening and monitoring patients with SMA. Optimizing its parameters further, can boost its clinical effectiveness in managing SMA, particularly in region with limited resources.

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Safety and efficacy of intravenous onasemnogene abeparvovec in paediatric patients with spinal muscular atrophy: findings from the phase 3b SMART study

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Clinical trials have demonstrated safety and efficacy of intravenous onasemnogene abeparvovec in patients with spinal muscular atrophy (SMA) weighing <8.5 kg. SMART was a phase 3b study to evaluate the safety, tolerability, and efficacy of intravenous onasemnogene abeparvovec over 52 weeks for patients weighing ≥8.5 to ≤21 kg.

Of 24 enrolled patients, 7, 8, and 9 were in the 8.5–13 kg, >13–17 kg, and >17–21 kg weight groups, respectively. 19 discontinued prior nusinersen; 2 discontinued prior risdiplam; 3 were treatment-naïve.

No study withdrawals or deaths have occurred. All patients had ≥1 TEAE; 15 (62.5%) had ≥1 SAE; 7 (29%) had SAEs related to onasemnogene abeparvovec. 20/24 patients (83.3%) had aminotransferase elevation adverse events; all cases were asymptomatic and managed with prophylactic prednisolone. Transaminase elevations (all Grade 1) were ongoing in 14 patients at the end of study. No bilirubin elevations or Hy's law cases were observed. Steroids were used over a median of 175.0 days. Transient thrombocytopenia was reported in 17/24 patients (70.8%); all resolved with no reported bleeding events. 3/24 patients (13%) had cardiac AEs (all unrelated to onasemnogene abeparvovec). No events of thrombotic microangiopathy or dorsal root ganglionopathy were observed. Frequency/severity of AEs were similar across weight groups. Motor function (RULM and motor milestones) was maintained or improved for most patients. Mean (SD) change from baseline at Week 52 was 2.0 (4.0) for RULM. By Week 52, three patients achieved standing with assistance, one patient achieved independent standing, and one patient achieved walking with assistance.

The safety of onasemnogene abeparvovec in SMART was consistent with previously reported findings. Most patients experienced transaminase elevations with no difference between weight groups; all cases were asymptomatic and managed with steroids. Most patients maintained or improved motor function, suggesting clinical benefit of intravenous onasemnogene abeparvovec for heavier patients with SMA.

P123

24 months of post-Zolgensma clinical follow-up in a child with Down syndrome and spinal muscular atrophy type I

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Firstborn of a non-consanguineous couple with Down syndrome (47, XY+21) with signs of central and peripheral hypotonia in the first weeks due to suggesting comorbidity for these clinical signs. Further investigation led to the comorbid diagnosis of Down Syndrome (DS) of spinal muscular atrophy (SMA), identified homozygous deletion of the SMN1 gene and two copies of SMN2. At 6 months he started treatment with Spinraza which was discontinued after his 7th dose, at 23 months and 21 days of life when he received treatment with Zolgensma.
This study describes the experience with SMA-modifying therapies in a patient with Down syndrome and SMA type I.

At six months of age, before the start of Spinraza his score was four points on the Chop Intend scale and after 7 doses of Spinraza, the day before gene therapy he scored 16 points on the same scale. 24 hours before the infusion of Zolgensma prednisolone was administered at a dose of 2mg/kg/day, considering that in the previous history of the® patient there was a report of transient increase of transaminases using Parsley tea prescribed to decrease sialorrhea. Three days after the Zolgensma infusion, he had an episode of fever (38ºC). The lowest platelet count in this period was 182,000 on the 7th day and the highest liver enzyme levels were obtained on the 41st day post-Zolgensma when GOT was 196IU/L and TGP was 307IU/L. Prednisolone was discontinued after 3 months and 25 days.
After gene therapy there was a gain in the chop intend score reaching a score of 24. at 4 years of age when he holds his head in position for 7 seconds, he stays out of the noninvasive ventilation (NIV) at intervals of up to 15 minutes a few times during the day.
In relation to weight-height growth the WHO curve escapes the upper standard deviations for children with DS, in the curve of typical WHO children it reaches the 95th percentile for weight and length.

The importance of this report lies in the fact that there is no description of the use of these therapies in children with Down syndrome and it was a very difficult decision for the medical team that exposed all the risks that we were exposing the patient. After all the medical considerations and in the absence of a contraindication to the use of the therapies, the decision of the parents was accepted, and they signed a consent form where in addition to other situations were clarified questions about the immune-mediated hepatotoxicity of gene therapy and that was very worrying because children with DS may in theory have a higher risk than other children. We consider that the adverse effects in this case did not deviate from the standard and that the adjusted dose of the systemic corticosteroid (2mg/kg/day) was decisive in the satisfactory control of aminotransferase levels. The 24-month clinical follow-up of this child showed that the therapy was safe and that its efficacy did not differ from the studies published in children of the general population.

P124

Development and validation of a clinical outcome measure for adolescents and adults living with SMA. SMA-LIFE STUDY

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There is an urgent need for assessing disease progression in adolescent and adult patients with Spinal Muscular Atrophy (SMA), due to the limited sensitivity to detect subtle changes with current scales. This study aims to develop and validate a new clinical outcome measure or took it to assess the functional status of adult SMA patients.

A panel of experts (neurologists, physiatrists and patient representatives) reviewed several existing clinimetric scales and PROs and agreed on the key dimensions needed to be assessed. After this revision they designed a questionnaire of 51 items assessing functional status from a clinical perspective in 7 key dimensions (bulbar, respiratory, axial, lower limbs, upper limbs, fatigability and other symptoms). Moreover, patients will be asked for the order of importance of each dimension following the PROOF questionnaire, so that the most important areas for each patient are considered when interpreting the global score. This will be combined with other quantitative measurements assessing 4 clinical dimensions (body mass index, forced vital capacity, Myopinch and 6-minute walk test) to make up the toolkit. This prospective, non-interventional study is being carried out at 5 centers in Spain and includes patients aged 16 years or older with a confirmed diagnosis of SMA 5q (biallelic mutation of the SMN1 gene). Physicians will administer the toolkit at 3 moments (baseline, 12 months, and 24 months). Additionally, data from other questionnaires and scales will be collected. The recruitment started on January 10th 2023 and will finish on October 31st 2023 with a target recruitment of 100 to 120 patients. In November, an interim statistical analysis will be performed to assess psychometric properties of the toolkit by applying Rasch analysis, based on the item-response theory, and statistics of classical test theory. Psychometric properties such as goodness of fit, dependency, reliability, and construct validity will be assessed, along with sensitivity to change. Also, during the study, other secondary endpoints will be evaluated including tracking patients' functional status over time, evaluating caregiver-assessed daily living assistance needs, validating a telematic application for the "toolkit," and comparing its sensitivity to changes against existing measures.

The final tool will consist of a questionnaire evaluating 7 key dimensions and including only those items selected after Rasch and psychometric analysis, a questionnaire including patients' preferred dimension, and 4 quantitative measurements assessing 4 clinical dimensions. The tool will provide an overall score and a score per dimension. Results of the usability, test-retest reliability, Rasch analysis and construct validity will be presented together with the final version of the questionnaire.

This study will provide a new easy-to-use assessment tool designed for and validated in adolescent and adult SMA patients. This tool will improve monitoring of disease progression, adapting to the unique needs of this patient population in both clinical practice and research, in the context of emerging disease-modifying therapies for SMA.

P125

Dynamic MR spectroscopy to detect early outcome of genetic therapies in Spinal Muscular Atrophy

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Identifying responders of novel genetic treatments before or shortly after start of treatment is an important challenge to minimize patient burden and maximize treatment cost-efficacy. Therefore, sensitive outcome measures that can detect the earliest effects of the treatment for SMA are needed. Here, we investigated the clinical utility of a novel diagnostic upper-arm exercise platform to detect early treatment effects on motor function via 7T phosphor magnetic resonance spectroscopy (MRS) readouts. We hypothesized that any metabolic consequences of positive treatment response at muscle level occur after 2 months and precede changes in muscle strength. Twelve patients with SMA (seven non-ambulatory) starting SMN suppletion treatment were included. Patients were studied at baseline, 2 months, and 10 months after treatment onset. Dynamic MRS data were collected from the biceps and triceps brachii muscles during concentric work at 80% of maximal voluntary contraction (MVC) force at baseline and after 2 months. Primary outcomes were changes in phosphor creatine (PCr), inorganic phosphate (Pi) and pH during exercise and their recovery rate constant. MVC force was measured at baseline and after 10 months using isometric dynamometry. The patients performed the exercise protocol in the MR scanner using the innovative platform. We collected 10 complete longitudinal datasets for the biceps muscle MVC force versus 8 for biceps MRS to conduct a preliminary test of our hypothesis. For weaker triceps muscle these numbers were 8 and 4, respectively. Median MVC of the biceps and triceps muscles remained stable in these patients over 10 months. Individual changes in muscle strength of the triceps after 10 months were positively correlated to changes in PCr recovery after two months ($r = 0.71$, $p = 0.03$). Associations between biceps muscle strength and MRS parameters were not significant.
In conclusion, this first test of a novel diagnostic upper-arm exercise platform in SMA patients suggests dynamic MRS readout of muscle metabolic state variables may allow early detection of treatment response. Further validation of this platform will be necessary in a larger cohort study.

P126

Data on safety and efficacy of Risdiplam treatment in a small apulian cohort of adult 5q spinal muscular atrophy

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Risdiplam is an oral small-molecule drug recently approved for the treatment of Spinal Muscular Atrophy (SMA). It increases the functional SMN (survival motor neuron) protein by modifying pre-mRNA splicing of the SMN2 gene. The aim of the study was to investigate the safety and efficacy of Risdiplam in our adult cohort of SMA patients.

The inclusion criteria were a clinical and molecular diagnosis of SMA2/SMA3, SMN2 copy numbers, availability of clinical data, and specific motor scale assessments [Hammersmith Functional Motor Scale Expanded (HFMSE), Revised Upper Limb Module (RULM), six-minute walking test (6MWT)] at treatment baseline, after six months (T6), and one year (T12).

We included 18 patients (9 SMA 2 and 9 SMA3), with a median age of 41 years at the first administration (IQR 37,7-48,6). Only 4 of them were walkers. HFMSE significantly increased from T0 to T6 (median values: 2, IQR: 0,25-12,75vs 3, IQR: 0,25-15,25 , p=0.027) and from T0 to T12 (median values: 2, IQR: 0,25-12,75 vs median values: 5, IQR: 1,25-16,75, p:0.033). The RULM did not show significant improvement from T0 to T6, but a significant improvement was observed at T12 (median values: 6,50, IQR: 15,50-21,25vs 13,50, IQR: 19,50-24,25, p=0.016). No changes in the 6MWT were detected at T6 or T12 in walking patients. Ten patients (55,6%) were classified as responders at T6, and 8 out of the 14 patients evaluated at T12 (57,1%%) were also classified as responders. Among all demographic and clinical variables, the number of SMN2 copies was independently associated with clinical improvement at T6 (p=0.023) and T12 (p=0.045). No severe adverse events were reported.

The natural history of types 2 and 3 SMA involves disease progression and continued loss of function. In patients with prolonged disease duration, early improvements are not expected. Notwithstanding the relative shorter follow-up, Risdiplam showed efficancy in stabilization or improvement of motor symptoms. This was demonstrated by the progressive improvements of HFMSE RULM. The mechanism of action of Risdiplam can explain the association between SMN2 copies and clinical improvement. Our data highlight the safety and efficacy of Risdiplam even in the first year of treatment, regardless of age, gender, functional clinical status at baseline, and SMA type. Number of SMN2 copies influence positively clinical improvement.

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Impact of SMN2-modifying treatments on the proteomic profile of plasma and cerebrospinal fluid samples from adult patients with Spinal Muscular Atrophy Type II and III

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Spinal muscular atrophy (SMA) is classified into four clinical sub-types, depending on the developmental milestones that are reached: Type I (severe), Type II (intermediate), Type III (mild) and Type IV (adult-onset). There is no cure, but clinically approved treatments including Nusinersen and Risdiplam are available to improve outcomes, highlighting need for prognostic and pharmacodynamic response biomarkers for SMA patients with different severities. Most research to date, however, has focused on understanding treatment responses in SMA I, and there is a lack of knowledge around treatment responses in SMA II and SMA III.

Plasma and cerebrospinal fluid samples were obtained from the Northern Care Alliance Research Collection (NCARC) from patients pre- and post-treatment (6 months) with Nusinersen (n=5; SMA III; 18-54y) and plasma samples from Risdiplam treated patients (n=3; SMA III; 19-47y). Quantitative proteomics analysis using Sequential Window Acquisition of all Theoretical Mass Spectra identified statistically significant differences in the abundance of proteins identified from two or more peptides between matched pre- and post-treated samples. The datasets were interrogated using Ingenuity Pathway Analysis to determine the canonical, cellular and molecular pathways impacted by the *SMN2*-modifying treatments.

SWATH-MS analysis identified 3102 proteins in plasma and 3105 proteins in CSF samples, respectively. At 6 months post-Nusinersen treatment 21 proteins were differentially abundant within the Type III plasma samples vs levels at baseline ($p<0.05$), of which 15 were downregulated and 6 upregulated whilst 19 proteins were differentially abundant in the CSF samples with 11 being downregulated and 8 upregulated. In the Type III Risdiplam treated plasma samples, 23 proteins were differentially abundant at 6 months vs baseline levels ($p<0.05$), of which 14 were downregulated and 9 upregulated. No protein was found to be similarly dysregulated in all datasets, but PLD4, a 5'-3' DNA exonuclease, was decreased post-Nusinersen treatment (0.18, $p=0.04$) and increased post-Risdiplam treatment (3.25, $p=0.03$). Bioinformatics analysis identified several significantly enriched molecular and disease functions in all datasets, including 'cell morphology' and 'neurological disease' whilst others, e.g., 'protein trafficking', were only enriched in CSF samples.

This work provides an insight into the plasma and CSF proteome profile of adult SMA patients following *SMN2*-modifying treatments. In ongoing work, treatment responses in Type II SMA patients following treatment with Risdiplam is under examination. Future work aims to verify the differential expression of proteins of interest in a larger patient cohort and to validate the use of these potential biomarkers to guide therapy selection, patient stratification, or elucidating treatment-specific efficacy biomarkers for SMA.

P128

Describing the real world impact of treatment initiation on motor function achievement in individuals with spinal muscular atrophy in the United States

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Natural history studies of infants with the most severe form of spinal muscular atrophy (SMA) have shown that 90% of motor neurons are lost by six months of age. Therefore, early diagnosis and intervention is necessary to slow the destruction of motor nerve cells, which cannot be regenerated. Currently, there are 3 survival motor neuron (SMN)-enhancing FDA approved treatments for SMA in the United States (US). Clinical trials of SMN-enhancing treatments have shown that earlier treatment initiation led to better motor function outcomes in infants and children, as demonstrated by an increased proportion of individuals achieving motor milestones such as sitting without support and walking independently at time of data cut off. There is limited evidence evaluating the impact of early treatment initiation on motor function in real world settings. Therefore, the objective of this analysis is to describe the relationship between age at first SMA treatment and motor milestone achievement in a real world population with SMA in the US.

Data from two Cure SMA databases will be used. The Clinical Data Registry (CDR) is a database comprised of electronic medical record-sourced data from the US SMA Care Center Network and is linked to clinician-entered SMA-specific electronic case report forms. The Community Update Survey (CUS) is an annual survey completed by individuals with SMA or their caregivers. This analysis will include individuals with 5q SMA that reside in the US and participate in the CDR and/or CUS. Individuals existing in both databases will be de-duplicated. The cohort will be restricted to individuals who were 2 years old at the time of data collection and have a known current motor milestone achievement (11 options from head control to walking alone) reported in the CDR or CUS. CDR data from 2022-2023 and CUS data from 2021-2023 will be used.

Demographics and SMA characteristics will be presented for the individuals included in the analysis. Caregiver or clinician-reported highest current milestone achieved at the time of data collection stratified by age at first treatment (<30, 30-90, and >90 days) will be presented. The group treated before 30 days of age will be further stratified if sample size allows. Results will be stratified by *SMN2* copy number.

Data from this analysis will aim to provide additional real world evidence to illustrate the importance of early treatment initiation. This analysis is limited by its descriptive nature, sample size, and mix of caregiver and clinician-reported data.

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Treatment expectations and patient reported outcome measures in adult 5qspinal muscular atrophy patients receiving disease-modifying therapy: An observational cohort study

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Efficacy of disease-modifying therapy (DMT), nusinersen or risdiplam, in adult 5q-spinal muscular atrophy (SMA) has been recently established in real-world studies focusing on motor function outcomes. Nevertheless, DMT effects on extraneuronal disease manifestations have not been explored. Moreover, there is still a need for adequate assessment of treatment expectations and patient reported outcome measures (PROMs) following treatment in domains not captured by motor function scales. The aim of this prospective cohort study was to investigate treatment expectations and PROMs in adult 5qSMA receiving DMT.

PROMs examining fatigability, breathing and voice, sleep and rest, vulnerability, self-reported improvement or worsening along with Stanford Expectations of Treatment Scale (SETS) were prospectively assessed in all eligible 5qSMA patients older than 16 years monitored at MDA Hellas Neuromuscular Diseases Unit in Thessaloniki prior to DMT initiation and every six-months of treatment.

507 completed questionnaires from 20 5qSMA patients were collected during a median follow-up of 19-months (range 1 to 24) after DMT initiation. 14 patients (79% type 2 and 21% type 3, 7% ambulatory) with a median age of 30 years (range 18-57) received risdiplam while six patients (83% type 3b and 17% type 4, 67% ambulatory) with a median age of 40 years (range 22-54) received nusinersen. Based on SETS 64% of patients receiving risdiplam and 66% of those receiving nusinersen expressed a positive expectation of motor function improvement while 7% and 66% respectively expected disease stabilisation. Interestingly, 21% of patients who received risdiplam and expressed a positive expectation, had some concerns regarding treatment side effects. 79% of patients receiving risdiplam (median follow-up 24-months, range 4-24 months) and 33% of those receiving nusinersen (median follow-up 6-months, range 1- 18 months) stated improvement in muscle strength, mobility, and endurance, while no patient reported worsening following treatment initiation. 29% of risdiplam treated patients stated improvement in fatigue and endurance, 7% in breathing and voice, 14% in sleep and rest and 21% stated improvement in vulnerability questionnaire. Respectively, 50% of nusinersen treated patients stated improvement in fatigue and endurance, 17% in breathing and voice, 17% in sleep and rest and 33% stated improvement in vulnerability. In contrast, only one patient who received risdiplam reported worsening in fatigue and endurance which further improved during therapy.

PROMs are emerging tools assessing adult 5qSMA patients' expectations and perception of changes following DMT initiation. In our cohort a positive therapeutic effect was observed through self-reported outcomes. Further multicentre validation studies are needed before PROMs integration in clinical trials and every-day clinical practice.

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Evaluation of the molecular response to Risdiplam in SMA patients through the analysis of SMN2 transcript levels on peripheral blood

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The clinical response of patients to Disease Modifying Treatments (DMTs) is widely variable and so far only clinically evaluable. Also, the therapeutic outcome in very compromised patients cannot be easily assessed through clinical measures, making biomarker identification crucial.
In our hands and based on our previous experience with salbutamol, SMN transcript levels in whole blood (differently from protein levels) are reliably evaluated also in response to treatment.

We are performing the longitudinal assessment of SMN transcript levels in whole blood samples of patients treated with Risdiplam, to evaluate the molecular effect of the compound and possible clinical and molecular correlations. We are collecting patients' samples at each follow-up examination (baseline, 6, 12, 18 and 24 months) and are performing the following molecular studies: *SMN2*-full length (-fl), *SMN*-del7 and total *SMN2* (SMN2-fl plus SMN-del7, *SMN2*-tot) transcript levels by absolute real-time PCR.
The following outcome measures were used: forced vital capacity (FVC), Hammersmith Functional Motor Scale Expanded (HFMSE), CHOP-INTEND scale and Revised Upper Limb Module (RULM).

Preliminary analysis has been made on 11 adult patients (mean age: 27± 16 yo) treated for at least 6 months. In all patients, Risdiplam modified SMN transcripts in blood at 6 and 12 months but the mode of action of the compound was unexpected: we observed a significant increase in *SMN2*-fl transcripts (mean 2.63±1.56 and 2.20±0.99 folds at 6 and 12 months, respectively) but also a global increase in *SMN*-tot transcripts (mean 2.55±1.72 and 1.31±1.16, respectively); the fl/del7 ratio showed a variable trend (mean variation 2.05±3.59 and 1.13±2.17). The increase in *SMN2*-fl transcripts persisted after 24 months of treatment (mean 3.49±2.53; 4/11 patients); in these patients the variation of *SMN2*-fl transcripts and the fl/del7 ratio were 2.39±1.37 and 3.54±6.33, respectively.
We performed an exploratory evaluation of the clinical outcome for possible correlations with the molecular response but we didn't observe any significant difference.

Our preliminary data show that the molecular effect of Risdiplam can be assessed through the quantification of *SMN2* transcript levels in blood, and that all patients respond at the molecular level. Most patients showed an increase in *SMN2*-fl but also in *SMN2*-tot transcripts, suggesting that the compound could also lead to a global gain in transcription of the *SMN2* genes or to *SMN2* transcripts stabilization.
Regarding the exploratory analysis of the clinical outcome, the muscle performance remained substantially stable in all patients analyzed, with no consistent increase. However, these patients were very weak adults, as shown by the baseline mean HFMSE score (2.7±2.21), thus an increase in functional score was not obvious.

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Unraveling the role of high-resolution ultrasonography of peripheral nerves in adult spinal muscular atrophy

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There is a need for outcome measures to assess disease progression and treatments effect in adult spinal muscular atrophy (SMA). High-resolution ultrasonography (US) is an established tool in neuromuscular disorders but only preliminary reports in small SMA populations have been currently produced.

In this single center observational study, we aimed to investigate a wide range of peripheral nerves in adult SMA patients using US to detail *in vivo* nerve morphology, in view of the potential role of US as imaging biomarker.

Genetically defined adult (>18 y/old) SMA patients were enrolled. We performed nerve ultrasound studies at: 1) the median and 2) ulnar nerve at wrist, forearm, elbow, arm and axilla; 3) posterior interosseous nerve at forearm; 4) radial nerve at spiral groove; 5) musculocutaneous nerve, 6) brachial plexus at supraclavicular space, 7) C5, C6 and C7 roots after leaving transversal processes, and 8) vagus nerve. At lower limb, 9) fibular nerve at fibular head and popliteal fossa and ankle; 10) tibial nerve at the ankle and popliteal fossa; 11) sciatic nerve at proximal thigh; 12) sural nerve at the distal calf. Results were compared with aged and sex matched controls, but also with and patients affected by amyotrophic lateral sclerosis (ALS) and sensory-motor diabetic polyneuropathy (DSPN), using two-sample Wilcoxon rank sum test (Mann–Whitney) test.

Thirteen adult SMA patients (1 SMA type 2, 12 SMA type 3; 6 females; 5 sitters and 8 walkers; disease duration 34.7±15.5y) were compared with 25 healthy subjects, 14 ALS patients and 26 patients with DSPN. The nerve cross-sectional area (CSA) of the C5, C6 and C7 nerve roots and of the vagus nerve were smaller in SMA versus the other groups. Only in SMA patients the upper arm nerve trunks were significantly thinner compared with the forearm, with conserved fascicle density (FD). The FD was significantly related to the muscular-US echogenicity at Heckmatt scales and to the MRC-strength scale ($p<0.0001$); both CSA and FD were lower in *sitter* versus walkers ($p<0.0001$). CSAs in the lower limbs were thinner than healthy controls but did not differ with the other patients (p 0.55).

We observed for the first time a reduction in proximal vs distal CSA in adult SMA patients, differently from what is commonly observed in other NMDs and neuropathies. The reduced CSA associated to maintained FD, compared to controls, might suggest a motor neuron/peripheral nerve development impairment in SMA patients.

Nerve-US was able to identify microstructural features in SMA patients and could be considered a candidate tool for longitudinal studies.

P132

The Spinal Muscular Atrophy Health Index (SMAHI) as a measure of disease change over time

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The Spinal Muscular Atrophy Health Index (SMAHI) is a disease-specific, patient reported outcome measure questionnaire designated to estimate personal perception of disease burden in patients 12 years and older. The SMAHI consist of 107 items, assessing 14 symptom domain (subscales); the symptoms and issues reported are the most frequent in patient with SMA and of high impact on patient's daily life. A high score obtained on a scale indicates that a particular domain or function impact on patient's burden. The aim of this study is to assess the ability of this questionnaire to detect changes over time, as perceived by the patient, considering the introduced treatments and the disease progression.

Patients with SMA were recruited in NEMO Clinical Center from 2018 to 2023. The SMAHI is administered every patient visit; for this study the first administration (T0) and the latest one (T1) were considered. For each patient, demographic and clinical characteristics in terms of age at evaluation, sex, SMA type and ambulation, time from disease onset and type of treatment were considered.

A total of 69 SMA patients (4 type I, 36 type II and 29 type III) were enrolled (mean age at evaluation: 31.42 yrs ± 14.61), male/female ratio of 1.09 (36/33). Of these, 29 patients were non-sitters (42.03%) at first evaluation, 34 patients were sitters (49.28%) and 6 patients were walkers (8.70%). The mean time between first and last observation was 35.51 months ± 17.82. Overall the analysis shows that SMAHI total score significantly improves over-time (Δ = - 0.14 points/months ± 1.94, p=0.0495) although with a small Cohen's d effect size. Considering the changes over-time in terms of points per months in the SMAHI subscores in the overall population, there was a mild perception of improvement in shoulders or arms function (problems with shoulders or arms subscore Δ = - 0.34 points/months ± 3.34, p=0.0248).

Although preliminary, our results suggest the SMAHI to be stable over-time when considered for the general cohort with a trend of improvement for single subscores (e.g. upper limbs).
In order to explore these preliminary results, further analyses are ongoing to correlate the changes over time of SMAHI specific subscores with the changes in functional motor scales used for the clinical practice.

P133

The integration of PROMs and clinician reported data: A holistic approach to characterise disease burden and treatment impact

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The UK SMA Patient Registry collects patient-reported outcome measures (PROMs) from individuals living with spinal muscular atrophy (SMA) in the United Kingdom and Ireland. In 2022, PROMs collection was introduced in the registry to supplement clinical and genetic data held therein. PROMs capture the perspectives of adults and caregivers of young people living with SMA about the impact of their condition and treatment, their quality of life and activities of daily living. Importance of the patient voice is increasingly recognised and valued. Currently, SMA therapies Nusinersen and Risdiplam are available in the UK via managed access agreements (MAAs). The collection of clinical and patient-reported data will inform review of treatment impact by UK regulatory authorities.

In collaboration with clinical networks Adult SMA REACH and SMA REACH UK, the registry aims to collect PROMs data of 100 Nusinersen and 100 Risdiplam patients. PROMs will be aligned with Adult SMA REACH and SMA REACH clinical data, anonymised, analysed and submitted to regulatory authorities for consideration as part of the treatment MAAs.

Registration in the UK SMA Patient Registry is patient-initiated through a secure online portal. Patients are invited to complete questionnaires about their condition and PROMs: EQ-5D; Patient Global Impression of Change; SMA Independence Scale (SMAIS-ULM); and a free-text box.

Enabled through patient consent and data sharing agreements, patient-level PROMs data is shared with each patient's SMA REACH clinic and with the SMA REACH coordination teams. In clinic, the data informs patient care. At project coordination level, PROMs are aligned with clinical data collected by SMA REACH.

The registry has 642 participants: 443 adult (16+years); 199 paediatric (<16years). PROMs have been completed by 205 adults and by the caregivers of 80 paediatric patients. The fraction of PROMs able to be aligned with Adult SMA REACH and SMA REACH clinical data is growing and will be presented.

The UK SMA Patient Registry represents a well-defined cohort of individuals with SMA and is a valuable tool for the collection of SMA real-world data reported by treated and treatment-naïve patients. Expansion of the registry to collect PROMs supports UK SMA data collection and supplements Adult SMA REACH and SMA REACH clinical data, assisting in therapy evaluation by regulatory authorities.

P134

Using innovative Data Modelling methods to improve data quality: learning from Adult SMA REACH a real-world data collection study

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Adult SMA REACH is a Real-World Data collection study for adult Spinal Muscular Atrophy (SMA) patients across United Kingdom. The aim of Adult SMA REACH is to improve the knowledge about the impact of new treatments and improved standards of care on the natural history of the diseases but also to support the approval of Nusinersen and Risdiplam in UK under the Managed Access Agreement Programme (MAA).

The generation of Real-World data requires a standardization and systematic data collection generated during routine clinical visits and constant monitoring of data quality to ensure that the data reported to regulatory agencies (NICE and NHSE) meet the highest quality standards.

Adult SMA REACH includes data from 19 different sites with 370 patients and 1200 follow-up visits. The manual monitoring of data for quality check was time consuming and highlighted the need for innovative methods of data monitoring.

Innovative data modelling techniques enable data representation as a structured collection of interconnected entities, enhanced with metadata. This format outperforms conventional tabular representations, facilitating more profound and intricate analysis.

Using this methodology, we created an automated software for data validation, consistency checks, completeness analysis and treatment tracking. This enabled us to provide continuous, hands-free monitoring of evolving datasets. The software also allowed us to reveal systematic issues on some sites impacting data quality, allowing us to provide targeted and tailored assistance.

We plan to expand our work by standardizing a data-agnostic data modelling methodology, allowing for a quick and flexible adaptation to different project scenarios within the John Walton Muscular Dystrophy Research Centre, Newcastle.

P135

Towards the identification of biomarkers of disease progression and response to treatment in spinal muscular atrophy

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Survival motor neuron (SMN) 1 gene, whose loss or mutations cause SMA, has ubiquitous expression in the organism, where it critically regulates several developmental and housekeeping cellular pathways, like RNA metabolism and biogenesis of microRNAs (miRNAs). MiRNAs are key gene expression modulators, whose dysregulation contributes to neuromuscular diseases; they are stable in body fluids and reflect distinct pathophysiological states, acting as promising biomarkers (BMs). Growing evidence suggests that
intrinsic skeletal muscle defects contribute to SMA pathology and we already demonstrated that the expression levels of circulating muscle-specific microRNAs (myomiRs), miR-133a, -133b, miR-206 and miR-1, decrease under nusinersen therapy in pediatric SMA. However, their potential as BMs for clinical use in adult SMA patients has not been investigated yet.

To analyze the expression profile of myomiRs in serum of adult SMA patients before and during nusinersen treatment to investigate their role as potential non-invasive prognostic and pharmacodynamic biomarkers.

Serum was collected from adult (>18y/old) SMA patients. Total RNA was isolated from serum using miRNeasy Advanced Serum kits (Qiagen). Expression levels of myomiRs at baseline, and after 6 and 14 months of nusinersen treatment, and in age-matched healthy controls, were assessed by RT-qPCR using Taqman microRNA Reverse Transcription kit and specific primers. Motor function assessment was performed by the Hammersmith Functional Motor Scale Expanded (HFMSE). Data analysis was performed by Mann Whitney test and Spearman's correlation analysis.

Two type-2 and 14 type-3 adult SMA patients (8 female; 2 non sitters, 2 sitter and 10 walkers) were enrolled. We detected an upregulation of miR-206, -133a and -133b before treatment in serum of adult SMA patients compared to controls ($p<0.001$; $p<0.005$ and $p<0.05$, respectively). A common pattern of reduced
expression till normalization of miR-206, -133a, -133b was evident in SMA patients upon 14 months of nusinersen administration, till normalization. Of note, baseline levels of miR-133a positively correlated with motor function at baseline and after treatment.

This investigation supports the role of serum myomiRs as non-invasive BMs to monitor disease progression and therapeutic response also in adult SMA, laying the groundwork to individualize patient management in the clinical practice. Particularly, in the clinical setting, miR-133a could help identify those patients most likely to demonstrate a meaningful response to nusinersen therapy.

P136

Capturing bulbar function in an international cohort of treated SMA 1 patients by using the PFOIS/CEDAS and OrSAT

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Disease modifying treatments (DMT) have substantially altered the survival and motor function trajectory of patients with Spinal Muscular Atrophy Type 1 (SMA1). However, the effect of DMT on bulbar function remains under investigated. Various tools have been proposed to capture bulbar function in treated SMA1, including the paediatric functional oral intake scale (PFOIS), recently renamed Children's Eating and Drinking Activity Scale (CEDAS), and the Oral and Swallowing Abilities Tool (OrSAT).

To understand the complimentary power of these tools, we cross-reference two previously reported cohorts of Nusinersen treated SMA1 in the UK and Italy.

Two-year change in bulbar function (across the PFOIS and OrSAT scores) was considered, with data collected every 6 months from treatment initiation date. We analysed PFOIS scores for 20 patients in Italy and 24 patients in the UK.

The OrSAT scores and levels were available in 35 (80%) of the patients, 17 in the UK and 18 in Italy. The Italian cohort was on average younger at treatment than the UK patients (median 6 and 11 months respectively, $p<0.001$). In the Italian cohort, 60% of participants had SMA1b (disease onset before 3 months), whilst half of the UK cohort has SMA1c (disease onset between 3 and 6 months). In the baseline assessments, the correlation between the PFOIS and the proportion of age appropriate OrSAT items is 0.88. Of the 14 SMA1b patients who have a PFOIS>3 (total oral feeding) at baseline, only 4 retain it at the 2-year follow up. In the SMA1c's, a PFOIS>3 is retained in 89% (8/9) of patients. In the SMA1b and SMA1c cohorts, 93% (14/15) and 100% (6/6) of the patients are observed to have developmental acquisitions of speech (items 11: "Able to speak>1 syllable" and 12: "Able to speak correctly>1 word"), respectively. In the SMA1b cohort, 40% (6/15) of patients are observed to progress through weaning stages ("Able to swallow semi liquids (item 2), semisolids (item 3) and swallow solids (item 4)". For the SMA1b cohort, these five items represent the bulk of the change; items 1 and 5-10 show predominantly stability or decline in function. In the SMA1c cohort, if we exclude the acquisition of the speech items, most patients maintain their baseline function across the majority of items.

The PFOIS and OrSAT capture complimentary information on the effect of DMT on bulbar function. The acquisition of speech should be considered separately as it captures different aspects of bulbar function. These scales could provide invaluable information in the long-term assessment of pre-symptomatic treated patients. Developmental acquisitions, like progress through weaning stages that are captured in the OrSAT, could represent potential aspects to target with a rehabilitation approach as part of the standard of care for DMT treated SMA1 participants.

P137

UK Real-world longitudinal data collection and analysis in adult SMA: the Adult SMA REACH Data collection study

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Recent therapeutic developments have led to the approval of three Spinal Muscular Atrophy (SMA) treatments by the European Medicines Agency. This approval highlighted the urgent need to collect data to gain a better understanding in the impact of new drugs on natural history of the disease. Currently, in The United Kingdom, only gene therapy (Zolgensma) is approved, with Nusinersen and Risdiplam having conditional approval only available for patients via Managed Access Agreement.

The Adult SMA Reach Real-World Data Collection Study, conducted across 19 sites in the UK, collects patients' clinical data and functional outcome measures generated during routine clinical assessments. The project aims to provide an overview of the disease progression for treated and non-treated patients. The collection of data contributes towards the final approval of the two drug treatments, but also to academic research with broader objectives.

The project analysed data from 361 SMA patients, comprising of 1154 visits, on 23rd of August 2023. The cohort of patients is showing the following distribution in subtypes: 6, 147, 180 and 4 patients with SMA Type 1, 2, 3 and 4, respectively. Key findings include the mean age at which the optimal motor function is attained, which is 0.91 years for SMA Type 2 patients (n=91) and 1.65 years for Type 3 patients (n=126). Additionally, 39.3% of SMA patients (n=142) have experienced ventilatory support during the study period, with 18 patients subsequently discontinuing this intervention. Notably, symptom onset age exhibits a correlation with SMA type, with Type 3 patients demonstrating later onset and a higher proportion of sitters and walkers compared to Type 2. The present analysis comprised of 212 Risdiplam, 106 Nusinersen and 49 non-treated patients.

These findings, and further comprehensive analysis, provide valuable insights into SMA real-world data, disease progression and treatment-driven disease progression. The collected data contributes to optimising patient care and advancing SMA research.

P138

Early effects of gene therapy on motor performance in children with spinal muscular atrophy

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The introduction of onasemnogene abeparvovec gene therapy (OAG) offers new possibilities for the treatment of spinal muscular atrophy (SMA). In Hungary, the treatment is available from November 2019, and from June 2021 with state support, on the basis of individual fairness. The aim of our retrospective observational research is to investigate the effectiveness of OAG treatment in children with SMA. The study was conducted at the Bethesda Children's Hospital of the Reformed Church and the Pediatrics Center of Semmelweis University between November 2019 and September 2023.

The study included all symptomatic children with SMA who received OAG treatment at the two national centres until 31.12.2022. Assessments were performed before, 3, 6, and 12 months after OAG therapy. In addition to the mandatory medical examinations, the functional measurement consisted of monitoring changes in motor abilities using the CHOP-INTEND (The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders) and/or the HFMSE (Hammersmith Functional Motor Scale Expanded) scale. A 3-point increase in the scale is considered clinically significant.

Both the pre-therapy examination and the subsequent care and follow-up (FU) were performed by a multidisciplinary team. After the gene therapy, we started regular physiotherapy for the children from the fourth week on average. Therapies included specialized physiotherapy, occupational therapy, provision of assistive devices and parent education.

Of the 33 symptomatic children included in the study (average age 16.2 ± 9.37 months; the youngest 1.5; the oldest 33 months old), 26 were diagnosed with SMA1 and 7 children with SMA2.
Children tested with the CHOP-INTEND had an initial (pre-OAG) mean score of 35.48 (±11.62; n=31), which increased by an average of 10 points at 12 weeks FU (±5.13; min. 1; max. 23; $p<0.001$; n=30), 13.05 points at 6 months (±6.55; min. 3; max. 28; n=21) and 17.69 points at 12 months FU (±8.62; min. 3; max. 37; n=13).
The mean initial (pre-OAG) score for children assessed with the HFMSE test was 12.3 (±11.54; n=10), which increased by an average of 8.2 points at 12 weeks FU (±2.94; min. 4; max. 15; $p<0.001$; n=10), 11.63 points at 6 months FU (±3.96; min. 6; max. 17; n=8) and 14.57 points at 12 months FU (±6.21; min. 6; max. 22; n=7).
The motor performance improved significantly for the first FU visit and remained significant for the whole FU period for both tests. We also experienced further significant improvement for every FU visit compared to the previous one.

Literature and our own experimental research support the early effectiveness of single OAG therapy for children with SMA. Our measurements show that gene therapy is effective when supplemented with complex physical therapy care and parent education.

P139

Evaluating fatigue, fatigability and well-being in patients with spinal muscular atrophy: The Nordic Experience

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In the era of newborn screening and access to gene therapy, one could argue that the ethical issues surrounding the detection and treatment of SMA begin to fade. How is the ethical framework changing along with the changing landscape of SMA detection and treatment and how does this correlate to providing patient-centered care?

A Nordic scientific roundtable discussion and subsequent meetings took place in 2022 to capture and share the experience of Nordic countries, i.e. Sweden, Norway, Finland and Denmark, regarding the outcome measures used in the evaluation of current treatments for SMA as well as to highlight unmet needs. Participants were mainly healthcare professionals specializing in neurology, pulmonology and physiotherapy with expertise within the field of SMA. Here, we present our initial consensus results and recommendations.

Four major topics were highlighted, i.e. (a) unequal access to targeted therapies; (b) complexity of the therapeutic decision-making; (c) stress and fear of losing access to ongoing treatment; (d) uncertainty about the evolution of the disease and associated co-morbidities. Establishing treatment benefit was the common denominator in many of the ethical issues discussed. The role of functional assessment tests in showing treatment benefit is major, making patient performance on these tests a great source of anxiety for the patient and the family. New survivors and older patients with SMA constitute a previously unknown group of patients with a new panorama of co-morbidities. This disease evolution inevitably poses new challenges to the evaluation and decision-making process.

There is a need for open and clear communication between the clinicians and the patient/family to acknowledge the ethical challenges arising with the evolution of the disease and involve the patient in his/her care. Harmonizing the criteria for patient identification and access to high-cost therapies is essential. Further work is needed to increase awareness regarding the therapeutic decision-making, to ensure informed decisions and patient-centered planning of care and rehabilitation.

P140

CUIDAME: Longitudinal analysis of the Spanish registry of patients with spinal muscular atrophy

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Natural history of SMA has changed due to improvement in treatment and better disease management. However, the real-world evidence on the impact of new treatments remains unknown. The aim of CuidAME Project is to collect Longitudinal Clinical Data of Spanish SMA patients to generate Real-World data.
The SMArtCare platform is used to collect retrospective and prospective data of SMA patients, regardless of their treatment regimen, in approximately 30 sites and followed for 5 years. The data is collected during routine clinical visits and updated every eight months. The data collected includes the main characteristics of the onset and evolution of the disease, genetic diagnosis, treatment and motor function assessments.
On August 23rd 2023, 24 centres had included 399 patients in the study; 22% (n=88) type 1; 46% (n=182) type 2; 30% (n=118) type 3; 1% (n= 2) type 4 and 1% (n=5) are presymtomatic patients. 49% (n=196) of the population are adults (≥ 16 years old).
In terms of treatment, 83% of the total population is on some disease-modifying treatment and 17% have been treated with best supportive care only. Longitudinal data measuring different outcomes will be presented at the time of the congress.
CuidAME is currently the platform containing the largest harmonized and standardized SMA clinician reported data in Spain. There is still no national new born screening (NBS) for SMA, and therefore the low number of presymptomatic patients is striking, emphasizing the need to implement NBS as soon as possible. CuidAME promotes national and international collaboration between centres and registries expanding the knowledge on SMA.

P141

Pain in spinal muscular atrophy patients

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Patients diagnosed with spinal muscular atrophy (SMA) frequently experience chronic pain, increased fatigue and impaired daily living activities. This study aimed to investigate the prevalence, clinical characteristics and demographic data of chronic pain in SMA patients.

Chronic pain prevalence, clinical features, motor functions, and the characteristics of the pain felt by the patients who were followed up with a diagnosis of SMA type II and type III at Eskişehir Osmangazi University Faculty of Medicine between June 2023 and September 2023 were evaluated. About pain status parameters; Pain intensity, frequency, duration, location using a body map, and factors that aggravate and alleviate pain were reported.

A total of 13 patients were included in the study. Nine patients were being followed up with a diagnosis of SMA type II (mean age 117.3±50.2 months) and four patients were diagnosed with SMA type III (mean age 148.5±51.9 months). The prevalence of chronic pain in type II and III patients was 88.8% and 75%, respectively. Pain intensity in SMA patients was mild, but the pain usually occurred intermittently, once or a few times a month, mostly in the back and lower extremities. It was determined that stretching and posture disorders increased the pain the most during physical therapy, and the distraction strategy was most frequently used as a method of coping with pain.

Pain should always be evaluated systematically in children diagnosed with spinal muscular atrophy.

P142

Longitudinal trajectory of the Revised Hammersmith Scale in nusinersen-treated patients with SMA type 2 and 3: A retrospective analysis of a paediatric cohort in the UK

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The Revised Hammersmith Scale (RHS) is a clinical outcome measure for people affected by Spinal Muscular Atrophy (SMA), aiming to capture changes in function in non-ambulant SMA type 2 through to ambulant SMA type 3 patients in the current landscape of disease modifying treatments (DMTs). The Revised Upper Limb Module (RULM) is an assessment for upper limb function in SMA patients.
The RHS and the RULM are used in real-world data collection studies and clinical trials. To date, longitudinal RHS data in a treated SMA population have not been published.

To report baseline characteristics and baseline and follow up RHS and RULM scores of nusinersen-treated children (0-18years) consistent with the type 2 and 3 phenotypes. We also describe the correlation between the two scales.

A minimum of at least two data points (baseline and follow up, 540 assessments) per patient were extracted from the national neuromuscular database (NND). Baseline assessments occurred within 3 months pre-or post-first nusinersen dose. Function was defined by the World Health Organization (WHO) motor milestones.

A total of 103 patients (60% male, median age 6.8 years) were included. All patients had baseline RHS scores; 48% SMA Type 2 and 62% SMA Type 3 patients had baseline RULM scores.
Fifty-six SMA Type 2 patients (61% male, 84% sitters, 16% non-sitters) were included; median age 4.4 years (range=1.4-16.2). Seven patients had scoliosis surgery and 10 patients required non-invasive ventilation (NIV). Median baseline RHS total score was 9.5 (range=0-30) and median RULM total score was 17 (range=2-28). The correlation between total RHS and RULM scores was 0.83 ($p<0.001$). The correlation between 288 longitudinal RHS and available RULM scores was also significant.
Forty-five SMA Type 3 patients (58% male, 38% sitters, 60% walkers) were included; median age 9.5 years (range=2-17.4). Two patients required NIV and seven patients had reported scoliosis surgery. Median baseline RHS total score was 35 (range=2-67) and median RULM total score was 28.5 (range=10-37). The correlation between total RHS and RULM scores was 0.87 ($p<0.001$). The correlation between 241 longitudinal RHS and available RULM scores was also significant.
Two pre-symptomatic patients (mean age 1.8 years, 50% sitters, 50% walkers). Baseline RHS total scores were 14 and 35.

The SMA cohorts described reflect the heterogeneity of SMA. With the introduction of DMTs, the use of appropriate scales is vital. In the SMA Type 3 cohort, no walker achieved full marks on the RHS and there was no floor effect except for one weak non-sitter. This demonstrates the minimal ceiling effect of the RHS. There is a strong correlation between the RHS and RULM at baseline and follow up. The RHS captures the spectrum of the disease severity. The longitudinal RHS and RULM scores will be reported in detail. Further analysis of the floor and ceiling effects in long-term treated patients is needed.

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Organisation of targeted therapy for SMA in Uzbekistan: Review for 2021-2023

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SMA is the most common genetic cause of infant mortality; without the necessary treatment and constant respiratory support, most patients with type 1 SMA die before the age of two years. To reduce the mortality rate from SMA and improve the quality of life of patients with SMA, it is necessary to create a multidisciplinary team of specialists for early diagnosis, conduct expensive gene-modifying therapy and rehabilitation of patients with SMA. In this regard, the role of the government and public health in organizing programs for medical and social support for patients with SMA is very important.

for the first time in Uzbekistan, a pilot project for gene-modifying therapy of SMA with the drug risdiplam was launched in December 2021, as a result of which Resolution of the President of the Republic of Uzbekistan No. 217 dated April 25, 2022 was adopted on measures to create a system for providing medical and social assistance and free delivery medicines for sick children diagnosed with spinal muscular atrophy. As the program is implemented, the number of children receiving gene-modifying therapy for SMA increases significantly.

Currently, the unified national register includes 158 children with SMA from 127 families, 22 (17.3%) families have consanguineous marriages, 23 (18.1%) families have 2 living children with SMA, 4 (3. 1%) families had 3 living children with SMA, in 15 (11.8%) families there were cases of death of children from SMA. Combination therapy (onasemnogene abeparvovec+ risdiplam) is used in 1 patient with SMA type I, change therapy (nusinersen→risdiplam) in 3 patients. The mortality rate of children with SMA included in the registry was 44 children (2021 - 19, 2022 - 15, 11 months 2023 - 10), of which 33 (75%) patients were unable to receive targeted therapy, 11 (25%) patients died while receiving gene-modifying therapy.

The introduction of gene-modifying therapy for SMA in Uzbekistan for 2 years has reduced mortality from SMA by almost 2 times, reduced the frequency of hospitalizations due to complications of SMA, and significantly improved the quality of life of sick children with SMA.

P144

Harbouring four *SMN2 gene* copies does not guarantee a milder phenotype: SMA Registry in Spain

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While a higher SMN2 gene copy number is generally associated with a milder SMA phenotype, it is an imperfect predictor of disease progression. This study aims to characterize the profile of patients with four SMN2 copies from RegistrAME

All data are from RegistrAME: the Spanish Registry of SMA

As of September 2023, 294 of 325 patients with genetically confirmed SMA 5q provided a copy number report; of these, 75 had two SMN2 copies, 186 had three, and 33 had four.
Of the 33 patients with four SMN2 gene copies, six had SMA type 2, 26 type 3, and one type 4. Ages ranged from 9 to 66 years, (median 34.36). Seven had had scoliosis surgery, six used non-invasive ventilation for part of the day, and 23 were receiving disease-modifying treatment.
According to functional status, three patients were non-sitters (all SMA type 2, aged 14 to 47 years), 15 were sitters, and 15 were walkers. From the walker group, three required a wheelchair outdoors, two could no longer climb stairs, and three had difficulty climbing stairs. Six patients walked short distances and six required assistance to walk.
Regarding hand function, three patients could not reach their mouth with their hands despite having useful hand function; eight could bring their hands to their mouth but could not raise their hands above their heads; seven could not rotate; and six could only partially rotate.

Patients with four SMN2 copies experienced the impact of SMA from a young age, with the severity of the disease worsening over time. Of the seven children with four SMN2 copies (aged between 9 and 17 years), four were walkers, two were sitters, and one was a non-sitter.
In the adult group, of patients aged between 20 and 38 years, seven were walkers and seven sitters; and, of those aged between 42 and 66 years, four were walkers, five sitters, and two non-sitters.

Patients with four copies of the SMN2 gene represent a heterogeneous group, presenting the full spectrum of motor phenotypes from non-sitter to walker across all ages. Over half the patients with four copies will not be able to walk or will lose the ability to walk at some point. Some will even lose the ability to sit up unassisted or have useful hand function. Motor decline can occur from a young age and is not only seen with disease progression in adulthood.
It is also important to note that the functional status of a walker is not equivalent to a healthy person, as a significant number of patients in this group will experience limitations in walking quality, limitations or inability to climb stairs, or the need for a wheelchair or other walking assistance.
Harbouring four copies of the SMN2 gene implies that some patients will develop a high degree of disability. Improved evaluation of the quality of the SMN2 copies together with other possible biomarkers or predictive factors are needed to assess disease progression in these patients.

P145

Early Treatment Experience and Outcomes of Patients with Spinal Muscular Atrophy in Kosovo

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Spinal muscular atrophy (SMA) is a rare, genetic, neuromuscular disorder characterized by progressive motor neuron loss, resulting in muscle weakness and atrophy. The development and access to disease-modifying treatments have dramatically improved motor function and treatment outcomes in SMA patients. Access to risdiplam and nusinersen in Kosovo was obtained last year for the treatment of genetically confirmed SMA patients.

It aims to analyze the early treatment experience in SMA patients and the early effectiveness of the chosen treatment options over time.

Patient hospital files were utilized to collect data for this observational analysis. Information pertaining to the treatment regimen, including medication type and administration route, was recorded. Medical histories, physical examinations, and any reported adverse events were also documented during routine patient visits as real-world outcome data.

We collected data from six 5q diagnosed SMA patients. 3 out of 6 (50%) patients had SMA Type 1 and the rest Type 2; with an average age of 5.4 years (ranging from 1.3 to 13.7 years). Two patients (33.3%) were receiving risdiplam orally once daily; one patient (16.6%) was undergoing intrathecal nusinersen treatment; one was ineligible for treatment due to severe impairments, contractures, and severe scoliosis; and two (33.3%) were eligible for treatment but it was unavailable. The early treatment experience of the 3 patients undergoing treatment with disease-modifying therapies has been satisfactory so far, with no drug-related adverse events observed so far. Over the past five months, these patients demonstrated stabilization of their condition, gained strength, and experienced an overall improvement in well-being. Although it is still early in the treatment process, there is optimism for future motor function improvements and better quality of life.

The initial treatment experience with SMA patients in Kosovo has shown promise, with no treatment-related adverse events and positive outcomes observed in terms of stabilization and strength gain. Continued monitoring and research are necessary to further understand the long-term effectiveness of the chosen treatment options and explore additional therapeutic interventions for SMA patients in Kosovo.

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SMA 1 children are growing up: A Journey that can be done!

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Spinal Muscular Atrophy is a rare neuromuscular disease of children that reduces movement abilities from birth. Pharmacological therapies have partially modified the disease course even if high levels of dependence remain. SMA 1 children need to use wheelchairs from the age of one year to discover their domestic environment and from 3 years electric wheelchair to achieve autonomy and participation in social activities and relationship in the outside world. Nevertheless, many families find it hard to accept the physical limitation of their children and limit their social interactions.
For 30 years SAPRE (Family Centered Service) have been proposing an "Early Parental Empowerment Program" (PEPE), with more than 1500 SMA children and their parents coming from all over the world, with the aim of increasing parent's knowledge and skills, to be able to effectively and safely manage their child's SMA disease complications at home. The project "SMA 1 children are growing up: a Journey that can be done" aims to allow young people with SMA to participate in activities often considered impossible due to both physical barriers and social attitudes. The main goal is to enable SMA people feel fully included in new and exciting experiences along with their classmates, friends, siblings and parents and to spread the culture of inclusion.

The project is divided into 3 main inclusive practical experiences every year, for around 40 SMA 1-2 childrens (age 4-14 years) and their families creating new interactions with local public and private institutions and associations, helping hundreds of people to live with a different mindset about motor disability.
"WHITE DOLOMITES SKI SAPRE" Mono-Dual Ski experience over 1.800 mt in DOLOMITES Mountain's snow "4 WHEELED VENICE" Children drive their electrical wheelchairs to discover one of the most inaccessible and outstanding cities in the world. "3 ITALIAN SEASON SMAWALK" Walking, Speaking, Knowing, Sharing, Watching, Cycling, Wheeling in electric wheelchair among SMA children, siblings and peer's parents, discovering Italian cycle paths, during 3 seasons (spring, summer, autumn).

SMA Children should have electric wheelchair as soon as possible to live their freedom to move, participate to community events and other of their local residence always more often. The difficulty of SMA children's parents to give the permission to use the electric wheelchair to their children from 3years age is due to the lack of acceptance of the diagnosis and incurability of the disease, slowing down and precluding autonomy that can be achieved.

The participation of the families in this SAPRE project has produced positive support among peers and the realisation of best practice that can be replicated independently throughout life. It has allowed social exchange and comparison between families. The project also improves the QoL of families and children by giving them the opportunity to practice activities leaving their comfort zones.

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Experiences of family members of children with spinal muscular atrophy during the COVID-19 pandemic

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Little is known regarding how families including a child with spinal muscular atrophy (SMA) have experienced life during the coronavirus disease 2019 (COVID-19) pandemic. In this study, we explored how parents and grandparents of a child with SMA feel the COVID-19 pandemic has affected their lives, particularly as regards medication and rehabilitation of the child.

Thirty-nine parents (24 mothers, 15 fathers), and 3 grandmothers, of 28 children with SMA, answered a web-based survey with closed- and open-ended questions. In addition, telephone follow-ups were conducted with seven of the parents.

The daily life of parents and grandmothers of children with SMA has been affected by the pandemic. The most prominent change related to social life and the children's access to care, e.g., physiotherapy, and personal assistance, which has impacted on the way of life for entire families. The pandemic has also had some impact on where the children receive nusinersen treatment. Few family members reported having tested positive for COVID-19. None reported that their child with SMA had been severely ill in COVID-19.

Given these results, it may be valuable to reach out to families of severely ill children when societies and healthcare systems open up, to find out how they have coped with the consequences of the COVID-19 pandemic and offer further support if needed.

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Phenotypic spectrum of three unrelated SMA Italian patients with a compound heterozygosity for a deletion and rare missense mutation in SMN1 gene

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Spinal muscular atrophy (SMA) is caused by mutation of the survival motor neuron (SMN) gene and in only the remaining 5% there is an intragenic mutation. We analyzed the phenotypic spectrum of three unrelated SMA patients with missense mutation (exon 7 c.840C>T) in SMN1 which causes its conversion to SMN2.

For the genetic analysis we used (MLPA) and (NGS) while for the clinical evaluation the (HFMSE) and the (RULM).

In our cohort of twenty-four SMA patients with molecular diagnosis we found three patients (12,5%; 2M/1F) carrier an allele with the deletion of exons 7 and 8 of SMN1 and the other allele with a rare missense mutation (exon 7 c.840C>T) which causes its conversion to SMN2. All 3 patients had 2 copies of SMN2. In addition, one of two male patients carries a large deletion in NAIP gene. This last patient shows the most severe phenotype. He referred the onset of symptoms at the age of ten months, and he acquired only the sitting position, so he was classified as SMA II. At last neurological examination at the age 36 years old he presented a severe scoliosis and his score at HFMSE is 14 and RULM is 17. The other two patients were classified as SMA III, but their clinical severity was very different: the male refers a delay of the motor milestones, moreover at the age of 13 he lost the ambulation. At the last evaluation at the age of 31 years he also presented a severe scoliosis and the HFMSE score was 18 while RULM score was 24. In contrast, the female patient showed a milder phenotype. She presented an onset at 8 years old with walking difficulty but currently the patient is still ambulatory and presents at the last evaluation (20 years old) her HFMSE score is 60 and RULM is 38. Finally, none of the 3 patients has breathing difficulties and only SMA II patient has minimal swallowing difficulties.

The analysis of our patients is interesting because in our cohort the rare point mutation that converts SMN1 to SMN2 seems to occur more frequently than described previously, in fact it is reported as a very rare variant (< 3%), furthermore there are no dedicated genotype-phenotype studies. Our study seems to demonstrate that there is the same phenotypic variability described for patients presenting the homozygous deletion. Male subjects are worse off than female ones. This data, in one of the 2 male patients could be explained by the deletion of the NAIP gene but the difference between the other male and the female remains substantially unexplained. Therefore, further studies are needed to evaluate the effect of gender on phenotypic expression.

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Descriptive analysis of the Spinal Muscular Atrophy population over than 45 years of age included in the CuidAME project

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Spinal Muscular Atrophy is a genetic, neurodegenerative disease characterized by progressive muscle weakness and atrophy leading to disability and paralysis. Knowledge of the natural history of the disease in the adult population is scarce, even more on the evolution in patients over 45 years of age with the advent of new therapies.

The CUIDAME project is an observational Spanish SMA population database where clinical data are collected every 8 months approximately through the SMArtCare platform. Here we present a descriptive analysis of the subgroup of individuals with SMA over 45 years of age included in the platform in August 2023.
42 individuals were older than 45 years at the time of recruitment, representing the 23.2% of the adult population (considered people ≥ 16 years old) included in the study, with a mean age of 53.8 years and 52% are female. Regarding SMA type, the 72% were type 3 (n=31), 22% were type 2 (n=9) and one patient was type 4. All but two participants had 3 or 4 copies of SMN2, 56% (n= 23) and 39% (n=16), respectively. In terms of treatment, 77% of the population was treated (26 patients with nusinersen and 5 patients with risdiplam), with 53 years old as a mean age of start treatment, whereas 22% (n=9) were under supportive care only, and one patient stopped nusinersen treatment. Regarding the therapeutic effect, no relevant difference in motor scales has been evidenced in treated patients compared to untreated patients, although there is a tendency to a lower score in the 6MWT in untreated patients (-16.5m). Four episodes of adverse effects have been recorded, none related to medications.
CuidAME is the Spanish SMA clinical data platform and provides relevant data on clinical practice and the impact of new treatments on the different profiles of population with spinal muscular atrophy. In patients older than 45 years old, there is a stability in motor scales and a tendency to worsen in the 6MWT in the untreated patients, without relevant side effects.

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The correlation of age and educational level with the quality of life of children with Spinal Muscular Atrophy in Indonesia

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Spinal Muscular Atrophy (SMA) is a rare neuromuscular disorder that causes muscle weakness. Little is known about SMA in Indonesia, including its impact on quality of life (QoL). QoL is a multidimensional concept that encompasses subjective evaluations of positive and negative aspects of life. The components of the QoL of children with SMA include physical, emotional, social, school, communication, and family resources. This study aimed to assess the correlation between SMA patient characteristics and QoL.

The Pediatric Quality of Life Inventory (PedsQL) assesses health-related quality of life in children aged 2-18. It includes a Generic Core Set for comparing healthy and ill children, and a Neuromuscular Module for disease-specific modules. This study quantitatively analyzed Indonesian SMA children's quality of life using Indonesian PedsQL GCS and NMM. The PedsQL GCS measures physical, emotional, social, and school functioning, while the PedsQL NMM assesses neuromuscular disease, communication, and family resources. Both instruments were filled out by the child and parents to evaluate the child's condition in the past month. Parents provided information such as occupation, education, income, and age, and the children reported their education, SMA type, and age.

The total number of respondents who filled out the questionnaires was 54, but only 47 completed the questionnaires. This study showed that 57.4% of the participants were female, aged between 5 and 10 years, with an average of 10.23 years old,72.3% were students, and 89.4% were SMA type 2. The average NMM score of the children was 54.11 ±14.37, while that of the parents was 53.66 ± 13.51. Meanwhile, the average of children's GCS score was 48.91 ± 12.73, while parents' was 53.45 ± 13.00. Pearson's correlation test revealed a negative correlation between children's GCS score and age (-0.224; sig 1-tailed 0.065; α<10%) and between parents' NMM scores and their children's age (-0.219; sig 1-tailed 0.070; α<10%). Students had higher NMM and GCS scores than non-students, as indicated by the sig 1-tailed 0.076 (α<10%) for children's NMM score and sig 1-tailed 0.062 (α<10%) for children's GCS score.

GCS scores decreased with age, indicating that older children had poorer QoL. This was likely due to older children feeling more responsibility for their life, and their disability becoming more limiting and burdensome for their parents as their weakness progressed. Parents' NMM scores decreased with age, with more complications as the impairment worsened. This could be due to inadequate SMA management in Indonesia because of low awareness. Children with SMA who attended school had higher NMM and GCS scores than did non-students. Socializing and interacting with peers makes activities more comfortable for these children.
QoL is individualized and influenced by many factors. Age and education level were linked to QoL in Indonesian children with SMA. Further research should explore other factors to gain a better understanding.

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High heterogeneity of *SMN2* structures and variants revealed by complete sequencing of the SMN locus in 448 SMA patients

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The scenario of spinal muscular atrophy (SMA) has changed radically in recent years with the approval of disease modifying therapies and the implementation of new-born screening in many countries. In this context, the study of the *SMN2* copy number (*SMN2*_CN) in patients becomes essential as a predictor of the SMA phenotype. However, patients with the same *SMN2*_CN can develop different phenotypes, indicating that this correlation is not absolute and that not all *SMN2* copies are equivalent. Some of these discrepancies may be explained by *SMN2* modifier variants, such as c.859G>C and c.835-44A>G, but these variants are very infrequent, implying that the complete genomic data of *SMN2* should be investigated.

We aimed to apply complete MLPA (P021) and an specific NGS method for *SMN* genes (Blasco-Pérez et al. 2021) to a large number of SMA patients (n=448) to generate a database capturing *SMN* region variability. First, our results regarding *SMN2*_CN, shows that more than half of the patients presented three *SMN2* copies (279/448; 62%), followed by cases with two (83/448; 19%) and four *SMN2* copies (75/448; 17%). Less frequent were cases with one (6/448), five (4/448) or six (1/448) copies. Second, we looked for the modifier variants of *SMN2*. In our group, 15 patients presented the modifier c.859G>C and, interestingly, all cases could be associated with *Smn2-859C.1* and *Smn2-859C.2* haplotypes (Blasco-Pérez et al. 2022), pointing to a common ancestral origin. The positive modifier c.835-44A>G was identified only in four patients. Third, the structure of the *SMN2* genes was analysed through paralogous sequence variants (PSVs), which differentiate *SMN1* from *SMN2*. The presence of *SMN2-SMN1* hybrids was detected in 103 out of 448 patients (23%). The study of the specific binding regions of *SMN2* targeted therapies nursinesen and risdiplam did not show differences among the 448 patients, indicating that these regions are highly conserved. Analysis are ongoing to determine a possible influence of further SNPs and SNVs in the expression and splicing process of *SMN2*.

The application of this NGS method, adapted to the complexity of the SMA region, makes possible to confirm *SMN2*_CN and to further explore *SMN2* variants and structures, the implications of which are under investigation. Collecting all this information from each SMA patient would greatly improve the knowledge of *SMN2* variability and the current genotype-phenotype correlation of the disease.

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Novel *SMN1* variants identified by NGS sequencing of the entire *SMN1* gene

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Spinal muscular atrophy (SMA) is a severe neuromuscular autosomal recessive disorder characterized by progressive proximal muscle weakness and atrophy. At molecular level, it is caused by loss of both copies of *SMN1* gene. Concretely, in about 95% of cases SMA is due to the homozygous absence of exon 7 of *SMN1*. Accordingly, only 5% of the positive cases present pathogenic variants in *SMN1* and nearly all of them are compound heterozygous patients including one *SMN1* deletion and one pathogenic variant. More than 80 pathogenic variants have been described in *SMN1* all over the gene but especially in exons 3 and 6.

We have applied MLPA (P021) and NGS (Blasco-Pérez et al. 2021) to study 23 unrelated patients with SMA phenotype and at least one *SMN1* copy. This approach allowed to confirm the molecular diagnosis of SMA in 15 patients (15/23; 65%) by the detection of previously reported pathogenic variants in *SMN1*. In addition, we have identified novel *SMN1* candidate variants in 8 patients (8/23; 35%), including four intragenic deletions, one missense and three variants predicted to affect splicing (one located in the splicing canonical sequence, one deep intronic and one synonymous variant in exon 7). The *SMN2* copy number and structure were also investigated in these patients, allowing to ascertain the pathogenic role to the different *SMN1* variants and to refine genotype-phenotype correlation.

Applying the combination of Sanger, complete MLPA and NGS methods, we have detected and validated novel *SMN1* variants that confirm the molecular pathology in both alleles of these patients. The identification of both biallelic alterations in a given patient is crucial for opportunity and decision of treatment with disease modifying therapies.

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Non-invasive assessment of respiratory pattern in Spinal Muscular Atrophy type 1 by Structured Light Plethysmography

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Spinal muscular atrophy (SMA) type 1 is characterised by severe muscle hypotonia leading to early respiratory failure in early childhood. Treatment options have become available, yet respiratory outcome measures in SMA type 1 are still limited.
Structured Light Plethysmography (SLP) has been proposed as a non-invasive, light-based method of assessing patients' breathing pattern. It can be used in patients <1 year, as does not require patients' cooperation. SLP measures the thoraco-abdominal wall movements by projecting a grid of light onto the anterior thoraco-abdominal wall. The aim of the study was to assess the respiratory pattern in SMA type 1 patients via SLP.

Cross-sectional study of consecutive children with SMA type 1 not requiring 24h ventilation/day referred to G.Gaslini between June 2016 to May 2017. All children underwent one-minute tidal breathing recording by SLP in supine position while self-ventilating in room air.
The *Respiratory rate* and the *Rapid-Shallow Breathing Index* (volume of air exchanged at each breath) were recorded by SLP.
The average contribution of abdomen and chest to each breath (*Relative Expired Abdomen%*, *Relative Expired Chest%*) and the severity of thoraco-abdominal paradox (*Phase Angle*) were also acquired. On the same day, motor functional assessment (CHOP-INTEND) was performed.

Nineteen patients were included at a median (IQR) age of 2.3 years (1.4-7.9). Eighty-nine percent of them required ventilatory support from a median (IQR) age of 10.5 (5.5-24) months.
Their respiratory pattern captured via SLP showed a raised median (IQR) respiratory rate per age of 33.5 bpm (26.6-41.7). A prevalent abdominal contribution to tidal breathing was found with a median (IQR) *Relative Expired Abdomen of* 77% (68-90) *vs Chest* 23% (10-32). Thoracoabdominal paradox was detected (median *Phase Angle* 48.70°) and its severity was found to negatively correlate with CHOP-INTEND (r -0.8, $p<0.01$).

SLP has been shown to be effective in capturing the respiratory features of SMA type 1, which are i) a rapid and shallow breathing, ii) a relative weakness of intercostal muscles of the chest with a prevalent abdominal contribution to each breath due to the relative sparing of the diaphragm, with the subsequent thoraco-abdominal out-of-phase paradoxical breathing.
This non-invasive tool will be useful in determining the role of disease-modifying treatments in SMA type 1.

P154

Move over, MLPA: Digital PCR as a cost effective, fast and accurate diagnostic tool for determination of copy numbers of *SMN1* and *SMN2* genes

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Spinal muscular atrophy is one of the most common lethal autosomal recessive disorders. For the diagnosis, prognosis, and management of treatment options; determination of copy numbers of the *SMN1* and *SMN2* genes are essential. Most affected individuals have a homozygous deletion in exon 7 of the *SMN1* gene and a wide variation in the number of copies of the *SMN2* gene. *SMN1* and *SMN2* genes, although each 20 kb in size, differ by only five nucleotides in total. This presents as a challenge in accurate determination of affected individuals by molecular techniques. Highly sensitive techniques are required for accurate determination of exon7 copy numbers of both genes. Traditionally, multiplex-ligation dependent probe amplification (MLPA) has been recognized as the gold standard for *SMN1* and *SMN2* copy number determination, making it the diagnostic tool of choice. The high cost, dependency on a sole source vendor, long procedure and a limited ability to scale, however, are factors that lead to delays in confirmation of diagnosis and initiation of treatment. Digital PCR (dPCR) is a modern variation of polymerase chain reaction which can quantify nucleic acids with unprecedented accuracy. It enables absolute quantification of the number of copies of the genetic target in the starting material. Digital PCR is capable of becoming a reference method for copy number variation determination assays, especially since assay time is only a fraction of that of MLPA while accuracy is comparable. Herein, we describe the clinical validation of a SMA dPCR assay.

Twenty nine anonymized leftover whole blood and dried blood spot samples previously tested with the MRC Holland SALSA MLPA Probemix P060 were tested with the Trimaris SMA dPCR Kit. The results were compared using the method comparison module of the Analyse-it Method Validation Edition.

Agreement between the two methods was 100%; *SMN1* 0cp (CI 64.9%-100.0%), *SMN1* 1cp (CI 80.3%-100.0%), *SMN1* 2cp (CI 81.6%-100.0%), *SMN1* 3cp (CI 27.0%-100.0%), *SMN2* 0cp (CI 42.5%-100.0%), *SMN2* 1cp (CI 64.9%-100.0%), *SMN2* 2cp (CI 80.3%-100.0%), *SMN2* 3cp (CI 74.7%-100.0%), *SMN2* 4cp (CI 52.6%-100.0%).

Rapid diagnosis allows earlier initiation of treatment, which is linked to more favorable outcomes. The Trimaris SMA digital PCR assay described here is an accurate, rapid and cost-effective method that is easily accessible to laboratories as a novel CE-IVD marked diagnostic assay for SMA.

P155
Impact of contractures on daily functioning in adolescents with spinal muscular atrophy: A qualitative study

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Exploring impact of contractures in adolescents and young adults (AYA) with spinal muscular atrophy (SMA) on daily functioning and participation and impact of received contracture management.

We included 14, non-ambulant AYA with SMA types 2/3 (10 females and 4 males), aged 16-30 years. Interviews focused on two topics: perceived impact of contractures on daily functioning and of previous contracture management. We used inductive thematic analysis for interview analysis.

In general, participants experienced muscle weakness to be more of a hindrance than contractures; they had adapted to their contractures over time. Participants considered contracture treatment useful when goals were meaningful and realistic. Participants mentioned that their perspective on contracture management would change in the light of a promise of improved motor function due to disease modifying treatment.

Despite the relatively low impact of contractures in comparison to loss of muscle strength, non-ambulant AYA with SMA should be informed on the potential impact of contractures and benefits and potential adverse effects of their management. This information can support the shared decision-making process. While respecting individual choices, it allows for incorporating interventions into daily life and promotion of daily functioning and participation when children with SMA are growing up.

P156
Multiple-setting, client-centered occupational therapy for a non-ambulatory SMA-T2 paediatric patient: A case report

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Therapies set in the patient's everyday settings have shown to be of great impact on the independence and function of physically disabled individuals. Non ambulatory spinal muscular atrophy type 2 (SMA-T2) patients face great challenges to be able to achieve as independent activities of daily living (ADL's) as possible. The aim of this poster is to show how a multiple-setting, client-centered intervention letting the individual choose which skills to work on whilst helping her prioritize between them with the help of continuous performance analysis, greatly helps make occupational therapy a motivational and functional tool, while assuring generalizable and meaningful outcomes for the patient.

The intervention is initiated by an at-home ADL performance analysis, shaped as an in depth one on one interview structured around the Child's Occupational Self-Assessment (COSA). From that initial session, four different ADL's are chosen and worked on in the form of task-specific training in by-monthly occupational therapy (OT) sessions at home and weekly sessions at the therapy center focusing on acquiring new skills or generalizing acquired skills to other settings by moving the intervention there. The skills are considered achieved when they are performed a set amount of times a week, three weeks in a row and are then swapped for other parts of the same ADL or into more challenging parts of the same skill. The child herself, measures the amount of times a week the skills have been performed by a magnetic and dynamic table of objectives. The COSA is filled and the interview is conducted again every 3 months to help the child prioritize which skills to work on.

All of the ADL related skills worked on have shown a great improvement. Toileting has improved from a complete reliance on external help to a complete independence on hygiene, the acquisition of much less aided transfers to the toilet less than 30 seconds long and the acquisition of completely independent transfers in under a minute. Shoe and sock dressing and undressing are being performed more than 3 times a week at home and twice a week (everyday) at the therapy center. Hair brushing and pony-tail making have improved from non-existent skills to being used more than thrice a week whilst being her preferred way of doing it and she is setting up the table more than 3 times a week.

The OT intervention has helped generalize skills developed in OT, as well as others trained in other therapeutic areas such as physical therapy, into many different everyday settings while progressively increasing the amount of times per week these skills are being used in detriment of more aid driven alternatives. Most of the skills trained have already become part of her everyday set of ADL related abilities. On the other hand, other widely used but least perfected abilities will continue to be worked on trying to reduce the need of external support and time strain.

P157

A monocentric, prospective, longitudinal study investigating the feasibility, accptability, safety and efficacy of an optimized rehabilitation for treated patients with SMA in United Kingdom

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Following the emergence of disease-modifying therapies and the transition to a more proactive approach to Spinal muscular atrophy (SMA) management, the natural history of this condition has changed. With the new phenotypes emerging, the importance of more regular, even daily exercise, was also highlighted, leading to an increased range of new approaches and possibilities of rehabilitation. Studies compared different techniques and/or showed the benefits of an unsupervised regular activity with innovative rehabilitation devices. However, the lack of controlled studies was frequently pointed out. Despite the publication of guidelines, it is widely reported by community physiotherapists across the UK that the current level of NHS funding cannot support the prescribed level of input. A recent survey conducted in the UK showed that although 100% of paediatric patients reported access to physiotherapy, 57% expressed a desire to be seen more frequently. Additionally, although 91% of the paediatric cohort had access to exercise, 44% reported limitations in access to strengthening activities and 35% noted limitations to access endurance activities. For these reasons, we aim to conduct a study to evaluate the feasibility, acceptability, safety, and potential therapeutic benefits of an adapted and individualized rehabilitation plan for treated patients with SMA of all phenotypes, namely the non-sitter, sitter and walkers with a focus on motor function. The aim of this project is to provide a goal-oriented rehabilitation, a closer follow-up of patients by increasing the access to hands-on physiotherapy, to increase the communication between tertiary care and community physiotherapist, and to provide access to innovative rehabilitation devices at home to reduce the burden on parents who are currently dealing with the rehabilitation on their own. If this programme is successful, it would offer a lifeline for patients and carers with limited access to physiotherapy support and serve as a proof of concept to support funding and provision for other Neuromuscular services across the UK. Additionally, we aim to involve physiotherapy students in the delivery of hands-on physiotherapy, thereby improving awareness of SMA amongst the future physiotherapy workforces. The study design will allow us to construct a Bayesian inference-based approach by comparing our cohort to natural history data. This will avoid the need to recruit a control group in our cohort and permits a smaller sample group which is a frequent limitation when conducting research in the field of rare diseases. Bayesian inference allows comparison of patients with the potential evolution that they would have had without treatment. The aim of this abstract is to present the ACE SMA study to the SMA community which should start inclusion in January 2024.

P158

Multidisciplinary intervention program before spinal surgery in spinal muscular atrophy - A case report

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The case is a 10-year-old girl with Type II Spinal Muscular Atrophy (SMA) who has been a candidate for spinal surgery with conventional bars. Since then, to the previously running Physical Therapy program, we add Psychology to create a multidisciplinary intervention program to prepare for the surgery.

The patient was diagnosed with Type II SMA when she was six months old and has been part of a multidisciplinary intervention program for a year. The program is run by the authors, and it includes both a great amount of individual intervention and a family-centered period.

Though the objective of the study is the pre-surgical multidisciplinary intervention, it must be pointed out that the intervention program will be modified depending on the moment and the particular needs of the patient and her environment.
During the pre-surgical phase, the girl had three sessions per week in the center, two devoted to Physical Therapy and one to Psychology. The objective is to maintain the global functionality, prepare the muscle-skeleton and respiratory systems, furnish strategies to cope with the situation and reduce anxiety.

In the structure of the Physical Therapy program, the abilities obtained previously to the participation in the multidisciplinary intervention program are maintained, and the aim is to improve the new abilities related to activities of daily living. The psychologist aids in the learning of new coping and anxiety management strategies.
Furthermore, this change in the way of coping with difficulties has allowed her to make progress in the acquisition of new complex functional abilities, due to an improvement in the patient's security and rationalization capabilities. The family has also been assisted. Their cooperation to spread these advances and learning processes has been fundamental.

This case proves how a pre-surgical multidisciplinary approach in such a complex case, both surgically and emotionally, can ease the patient's recovery, reducing the time and improving the quality of how the patient can fall back into her routines after the surgery.

In Psychology, the program can help reduce the high pre-surgical anxiety of the patient, the family and her closest environment, training in different psychological techniques. The Physical Therapy strength, flexibility and line-up trainings will help prepare the muscles for the surgery.

P159

SMA type 2 evaluation and functional testing - Case report

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SMA type 2 is diagnosed up to 15 months of age, people never walk, some of them can sit independently until a certain age, some can even stand with the help of orthoses or verticalizers. Respiratory problems develop later, and swallowing problems are also possible. In 99% of cases, they develop scoliosis, which, if not corrected surgically, can make breathing difficult and cause other complications. Lifespan is shortened (highly variable) but largely depends on proper care

To present the rehabilitation treatment with functional tests in a patient with SMA type 2.

case report of a female patient with SMA type 2, 35 years old, employed, uses an electric wheelchair and is treated according to a protocol with the drug Risdiplam for one year, prescribed by a specialist neurologist. She has a severe degree of scoliosis and contractures at the elbow, knees and hips. The functional tests with which she was tested are: MFM-32 and RULM, at two times, before the start of medical therapy and after one year. Measurements of the range of motion of the joints of the upper and lower limbs were also made by a physiotherapist. During that time, the patient performed home kinesiotherapy three times a week with the help of a physiotherapist.

From the analysis with the MFM32 questionnaire, the total severity of the motor impairment in the first examination was assessed as very severe, and in the second examination it was assessed as moderate. Regarding the RULM questionnaire, for the assessment of the upper limbs there is also an improvement, in the first test the rate is 31, and in the second it is 33. In terms of measurements, there is an improvement in terms of contractures.

our analysis proved that taking the specific therapy in combination with kinesiotherapy has an effect on the patients in terms of improving the motor function, the range of movements in the joints, and better quality of life of the patients.

Keywords: rehabilitation, spinal muscular atrophy, kinesiotherapy

P160

Evaluation of the physiotherapy service in an adult Spinal Muscular Atrophy (SMA) tertiary centre in the United Kingdom (UK)

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The Managed Access Agreement (MAA) for disease modifying treatments (DMT) for SMA in England started in 2020. At St George's Hospital (SGH), like most neuromuscular centres in the UK, there was no dedicated SMA clinic. Clinical expertise of the multidisciplinary team (MDT), which included an experienced neuromuscular physiotherapist, along with patient feedback in clinic has expanded the service to both fulfill the MAA and improve the multidisciplinary clinic. The aims of this project were to evaluate the impact of the service from the patient's perspective, focusing on the assessment process, understanding of the genomic conversation around DMT and the provision of a monthly online Pilates class to support muscle strengthening.

An online questionnaire was sent to all adults with SMA treated at SGH to evaluate the assessment process. Two follow up focus groups were held to explore the themes of the questionnaire in more depth. Additionally, the monthly online Pilates classes were evaluated through an online questionnaire.

Thirty-nine adults with SMA have been assessed at SGH since July 2020, with 28 currently under our care on DMT (Nusinersen (n=11); Risdiplam (n=17); age range: 18-71 years). One further person under our care was previously on DMT but has since stopped. Thirteen responses were received from the assessment questionnaire (44.8% response rate; Nusinersen (n=6); Risdiplam (n=6); previously on treatment (n=1); age range: 20-59 years). Genomic understanding of the DMT improved after initial assessment in 10 of the 13 respondents. Eight respondents had an initial video consultation with the neurologist and physiotherapist, and all found this either useful or extremely useful. Two follow up focus groups were held with a total of 5 participants (Nusinersen (n=3); Risdiplam (n=2)). Themes emerging from the focus group include improved knowledge and understanding of treatment after initial assessment and a feeling of being supported by the MDT. Mandated outcome measurement sessions were viewed as a useful way of benchmarking function. Eleven responses were received from the Pilates class questionnaire. Eight respondents either agreed or strongly agreed that the Pilates classes made them feel stronger and 53.9% of respondents reported that they do more strengthening exercises outside of the classes since starting Pilates.

This service evaluation of a single adult SMA service in the UK showed high satisfaction. Outcomes from the questionnaire and focus groups demonstrated improved genomic understanding of DMT and feeling well supported by the MDT. A monthly online Pilates class was well received and reported benefits included feeling stronger and exercising more regularly. This evaluation demonstrates MDT input, including strengthening exercises, along with better understanding of treatment options have the potential to improve patient wellbeing and self-management.

P161

Novel Management of Scoliosis in Young Severe Spinal Muscular Atrophy

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With new drug treatments for Spinal Muscular Atrophy (SMA), we are seeing an increase in life expectancy and improved motor outcomes for these patients, changing the phenotype of this condition. Despite this, all children under 5 years with severe SMA will develop scoliosis. Spinal bracing leads to improvements in sitting ability, head control, upper limb function and reduction in fatigue. There is however hesitancy, as spinal bracing has previously been thought to negatively impact respiratory function, with bracing in the seated position reported to cause reduction in tidal expiratory volume. There is also no agreed effective surgical method currently to manage scoliosis in young children without increasing risks of repeated anaesthesia, surgery and, potentially, reduced lung capacity. Development of severe scoliosis, however, also has negative effects in respiratory function of these patients, reducing their vital capacity. Disease modifying SMA drugs lead to SMA motor assessment improvements however the function of these children may still be limited by spinal posture and contractures. It's clear that new, proactive approaches are required to maintain spinal posture and joint ranges, to allow optimum functional benefits of novel therapies for these patients.

We have developed a guideline driven by the Neuromuscular MDT, involving the Neuromuscular, Respiratory and Spinal Surgery teams, with agreed parameters and monitoring by the different teams, and criteria met before children are selected for spinal bracing. We use individual bespoke SMA-CMPlite Braces for our SMA patients, using a 2-part CAD-CAM scan in clinic, providing an on-screen 3D model of the patient. The brace is unique, asymmetric for maximum curve correction, with an abdominal window to avoid restriction to breathing.

Our results show slowing in progression of scoliosis in braced patients compared to non-braced (P=0.0036; updated data accrued between submission and presentation will be included at presentation), with no adverse outcomes, including no increase in admission rate secondary to respiratory events, including infections. Good compliance (75% of patients wearing it in all upright positions regularly), improved function in head and upper limbs, improved sitting balance and endurance in children who wore the brace.

We have demonstrated the safety and efficacy of bespoke spinal bracing in patients with severe SMA, leading to significantly improved motor function and the slowing of progression of scoliosis. Spinal bracing did not worsen respiratory outcomes.

P162

Changing hip symptomatology and treatment options in pediatric SMA patients treated with disease-modifying treatments

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Over the last years, the clinical course of SMA and its survival rates have drastically improved due to innovative disease-modifying treatments such as nusinersen (Spinraza®), risdiplam (Evrysdi®) and onasemnogene abeparvovec (Zolgensma®). Due to these innovative treatments, patients often present with better physical outcomes. As a consequence, there is a general tendency towards more proactive and invasive treatments to further optimize the patient's condition. One key example pertains to hip pain due to hip (sub)luxations which is increasingly reported by patients. Multiple treatment options are available and can be applied such as infiltration of the hip with corticosteroids and local anesthetics, femur osteotomy or femoral head resection. Infiltration is often the first choice, yet its efficacy in pain control and symptoms is not always sufficient and transient. If needed, more invasive surgical options such as femur osteotomy or resection of the femoral head are performed. In this study, we will describe different individual cases who underwent treatment(s) for hip pain. We will look more deeply into pain experiences, treatment decisions, and outcomes in terms of physical functioning and daily life functioning.

We intend to include and describe at least 4 pediatric SMA patients suffering from hip pain. These patients have been treated with different treatments. We will describe and integrate experiences with respect to antecedents and pain intensity, treatment-related decisions, and the impact of treatment on physical functioning but also on daily live activities and well-being (e.g., school absenteeism, social activities).

Results and conclusions will be discussed in detail at the conference as we do not yet have any concrete results at this time. Future work is encouraged to systematically investigate the (pro-active) approach to hip pain and treatment, paying attention to medical as well as psychosocial aspects such as quality of life. A multidisciplinary approach is strongly recommended to provide well-advised and patient-oriented care.

P163

The effectiveness of a virtual physical therapy training program for carers of children with Spinal Muscular Atrophy type 1 hospitalized at home

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Physical therapy in pediatric palliative care is an integral component in the clinical management of children diagnosed with Spinal Muscular Atrophy (SMA) type 1. SMA has a wide range of clinical disease severity, this is reflected in the distinction between 4 types of the clinical classification system. Over the past decade, the wider body of literature has significantly improved standards of care among home care patients. Respiratory complications, such as hypoventilation and poor secretion clearance are major causes of morbidity and mortality in children diagnosed with neuromuscular disease. The proper handling of medical equipment within this patient population is imperative amongst caregivers as it involves specialized care techniques. An in-depth physical therapy educational training program is crucial in preparing carers and equipping them with the skillset and ability required in caring for children diagnosed with SMA.

A retrospective cohort study was utilized for this study. Educational competency prior to and after educational interventions was measured. The training program was carried out virtually over a 10-month period. Training modalities included basic physiology training, berthing patterns, respiratory problems, secretion management, dyspnea management and chest physical therapy incorporating the use of equipment at home. Additionally, the training program included musculoskeletal and deformity prevention.

A total of 24 participants caring for children diagnosed with SMA type 1 took part in the educational home care program over a 10-month period while receiving treatment from a physical therapist. The average theoretical assessment score improved following the training program. Participants self-rated their degree of knowledge using a Likert scale in each conceptual domain on a scale of 1 to 10. After the 10-month period, participants education improved by 60.3% (20.1% pre-training and 80.3% post training), this indicates a marked improvement from the original evaluation.

Carers supporting SMA type 1 patients at home demonstrated a high level of improvement in both knowledgebase and skillsets necessary to develop their role after completing the 10-month physical therapy educational program. An educational program can enhance abilities and improve self-reliance amongst carers caring for children diagnosed with SMA type 1. Moreover, this study demonstrated that the educational intervention had a positive impact on caregivers' self-perception, competence, and confidence.

P164

The "walk" with HKAFO orthoses in the treatment of Spinal Muscular Atrophy: "SMArtWalk"

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Evolution of the clinical overview of SMA following drug trials: increased the motor potential of patients, changed the scenario within the rehabilitation project. We designed a new HKAFO brace called Smartwalk that allows SMA type 1 and 2 patients to transition from static activity toward ambulatory activity. The Smartwalk orthosis gives the dynamism needed to learn a motor pattern.

Technical modifications to make SMArtWalk from Smalera: coxo-femoral joints for flexion-extension limited to 10-15°;tibio-tarsal joints for flexion-extension limited to max 10°;dynamic stylization of the foot;elastic rods to provide traction antagonistic to the action of the ileo-psoas muscles and the possibility of hip extension;integration with body FLEXA to achieve corrections in alignment and rotation; integration with rear support walker for learning a motor pattern that promotes autonomous walking. Functional criteria: absence of retractions and deformities that prevent reaching joint ROMs for tibio-tarsus, knee and coxo-femoral; absence of severe and non-reducible scoliotic curves; good trunk, shoulder and head control. Users already using Dubowitz or Smalera type orthoses with low pelvic grip; body mass within 30kg; ongoing drug therapy and clinical overview allowing partial recruitment of the main antigravity muscles deputed to walking; absence of cognitive delays.

Active search for autonomous balance. The operation of Tamarak joints installed at the tibio-tarsal allowed users to search for their own balance, allowing them to swing antero-posteriorly using displacement of the center of gravity within the established ROM. Complex challenge: learning a new motor pattern capable of producing autonomous advancement. Critical phase the throwing of the lower limb. Users with a higher degree of skill at the upper limb level passed this test more smoothly, because they were able with less effort to unbalance their center of gravity toward the 2 hemilaterals by being able to increase the distance between the swinging limb and the ground and thus zeroing the frictional force between the sole of the shoe and the ground itself. All users were able to gain autonomous balance in standing, and several of them took a few steps at reduced amplitude. With the SMArtWalk, we have made possible an increase in feasible functions in the rehabilitation setting

In all cases tested, the potential of SMArtWalk is not revealed during testing or delivery. The real efficiency of the system is revealed weeks later with due training carried out with trained personnel. The feedback received from families after this period is: increased stability and autonomy in using the Smartwalk, and user acceptance.

P165

ERK phosphorylation modulates autophagy deregulation in human Spinal Muscular Atrophy differentiated motoneurons

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Spinal Muscular Atrophy (SMA) is a genetic neuromuscular disease characterized by the degeneration of the spinal cord motoneurons (MNs), weakness, and progressive muscular atrophy. SMA is caused by the homozygous disruption of the *Survival motor neuron 1 (SMN1)* gene, resulting in deficient levels of the SMN protein. The precise intracellular mechanisms related to MN degeneration in the SMA condition are not fully understood. However, the impairment of some survival signaling pathways, such as PI3K/Akt and ERK MAPK, and the deregulation of the autophagy process have been described in several SMA models. In the present work, our objectives are: a) analyze the contribution of ERK MAPK pathway on SMN protein regulation in MNs, and b) investigate the role of ERK MAPK in autophagy deregulation in SMA cells.

We differentiated human induced pluripotent stem cell lines (iPSCs Control: GM23411*B, and SMA type 2: GM23240*B; from Coriell Institute) to MNs. Control and SMA iPSCs were differentiated to neuroepithelial cells, MNs progenitors (MNPI and MNPII), neurospheres, and finally MNs, as described. After 6 days in vitro (DIV) we treated cultured MNs with: a) the MEK inhibitor U0126 and analyzed SMN, protein and mRNA levels (Western Blot and RT-qPCR, respectively), and the autophagy markers LC3-II, p62 and mTOR phosphorylation (Western Blot); or b) the calcium chelator BAPTA-AM and analyzed SMN and LC3-II protein levels (Western Blot).

The pharmacological inhibition of the ERK MAPK pathway with U0126 reduced ERK phosphorylation and decreased SMN protein and mRNA levels in control and SMA differentiated MNs. Additionally, ERK inhibition induced a significant reduction of mTOR, LC3-II, and p62 autophagy markers in SMA MNs. Furthermore, the intracellular calcium chelator BAPTA prevented ERK hyperphosphorylation and reduced LC3-II levels in human SMA differentiated MNs.

Our results suggest that ERK MAPK pathway regulates SMN at protein and transcriptional level, and that ERK hyperphosphorylation observed in SMA MNs may contribute to autophagy deregulation in SMN-reduced MNs.

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P166

Analysis of neurite degeneration in cultured Spinal Muscular Atrophy motoneurons: Enhanced Fiji Macro for Degeneration Index Measurement in confocal images

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Spinal Muscular Atrophy (SMA) is a neurodegenerative disease characterized by a progressive loss of motoneurons due to a reduced Survival Motor Neuron (SMN) protein level. Before neuronal death, motoneurons present neurite degeneration. The quantification of this early-stage process becomes a valuable tool for evaluating the disease's progression. A common method for analyzing neurite collapse is calculating the Degeneration Index (DI). However, the drawback of this method is the accumulation of errors in this kind of measurement. We have adapted a previously published Fiji macro to objectively quantify fragmented neurites using confocal images to address this issue.

Control and SMA mouse and human iPSCs differentiated motoneurons were cultured and submitted to immunofluorescence protocol using an anti-beta-III-tubulin antibody. Immunofluorescence images were performed with the Olympus FV10i confocal microscope. The macro was validated by submitting the cells to different experimental conditions.

The Fiji analysis implemented is specific to confocal microscopy images from the Olympus brand. Furthermore, statistical analysis confirmed that the results obtained with the Fiji macro-based analysis were statistically equivalent to those obtained through manual analyses.

We have adapted a previously designed bioinformatics tool for quantifying motoneuron neurite degeneration with confocal images. This work introduces a precise and automated solution, mitigating measurement errors and significantly enhancing efficiency.

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Motor neuron pathology drive spinal circuit defects and phenotype of a mouse model for spinal muscular atrophy with respiratory distress type 1 (SMARD1)

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The group of spinal muscular atrophies can be subdivided into the classical proximal form, spinal muscular atrophy (SMA), and distal spinal muscular atrophy with respiratory distress type 1 (SMARD1). The main drivers of SMA are motor circuit defects including neuromuscular junction (NMJ) denervation, motor neuron death and loss of central premotor synapses. In contrast, little is known about the pathology of spinal motor circuits in SMARD1, which is caused by a deficiency of the DNA/RNA binding protein IGHBMP2. Most studies have been performed in the SMARD1 mouse model model "*Nmd*^{2J}". The mutant *Nmd*^{2J} mice exhibit vast motor neuron loss and NMJ denervation. However, it is neither clear whether spinal synapses are affected nor which cell type drives the neurodegeneration.

We applied immunofluorescence and confocal analysis to investigate motor circuits of the most widely used SMARD1 mouse model *Nmd*^{2J} by quantifying motor neuron death, muscle denervation and spinal synaptic loss. To get access to motor neuron function, we performed whole-cell patch-clamp recordings of motor neurons in spinal cords of *Nmd*^{2J} mice. Furthermore, we injected adeno-associated virus 9 (AAV9) into perinatal mice to selectively restore Ighmbp2 in motor neurons of *Nmd*^{2J} mutants. Spinal circuit pathology and motor behavior were then analyzed.

First we compared the pathology of "proximal" motor circuits consisting of the lumbar L1 spinal segment and its axial target muscles with "distal" motor circuits consisting of the L5 segment and distal muscles. We found coincidental α-motor neuron death and muscle denervation within the first two weeks of life which both extended to ~70% at 6 weeks selectively in distal motor circuits, matching the distal phenotype of *Nmd*^{2J} mice. Similarly, a selective 50% loss of premotor excitatory synapses (C-boutons and proprioceptive synapses) in distal motor circuits was present by 4 weeks when the first motor impairments became apparent. Electrophysiology recordings revealed hyperexcitability of *Nmd*^{2J} motor neurons, although proprioceptive synaptic transmission was significantly reduced. To identify the cause of motor circuit degeneration, we used a virus that conditionally overexpresses IGHBMP2 (AAV9-IGHBMP2fl/fl) upon Cre recombinase induction. As a proof-of-principle, co-injection of AAV9-IGHBMP2fl/fl with AAV9-Cre resulted in complete correction of the motor circuit pathology. By injecting AAV9-IGHBMP2fl/fl into *Nmd*^{2J} mice expressing a motor neuron specific Cre, we restored IGHBMP2 selectively in motor neurons. These mice also exhibited an almost complete rescue of the entire motor circuit pathology including motor neuron death, proprioceptive synaptic degeneration and motor phenotype of the *Nmd*^{2J} mice, demonstrating that motor neuron defects drive SMARD1 pathology.

Our findings link selective motor circuit pathology, including severe proprioceptive synaptic loss and motor neuron death, to the observed "distal" phenotype of *Nmd*^{2J} mice and SMARD1 patients. We develop a novel genetic-viral approach to implement cell type-specific IGHMBP2 expression and demonstrate that motor neuron drive SMARD1 pathology. These findings lay the ground for identifying novel disease markers and candidate therapeutic targets to ameliorate this incurable disease.

Acknowledgments

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Gemin3 reduction may contribute to neurodegeneration through NF-kappaB inhibition: Role in Spinal Muscular Atrophy pathology

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NF-κB pathway activation is required for neuronal survival and regulates Survival Motor Neuron (SMN) protein in cultured spinal cord motoneurons (MNs). In addition, Gemin3, a core member of the SMN complex, regulates NF-κB activation through the mitogen-activated protein kinase TAK1. In order to elucidate the link between SMN, Gemin3, and NF-κB pathway, we analyzed Gemin3 and NF-κB members, inhibitor of kappa B kinase beta (IKKβ), and RelA in mouse and human SMA models.

We used three human fibroblasts cell lines from two SMA patients and one unaffected control; MNs differentiated from human-induced pluripotent stem cells (iPSCs) obtained from two SMA patients and one unaffected control; spinal cord and quadriceps from the SMNΔ7 mouse model at pre-symptomatic postnatal day 5 (P5); and MNs isolated from the spinal cord of CD1 or SMA mouse E12.5 embryos. To characterize differentiated MNs, we performed immunofluorescence assay. Cultured cells were fixed after 6 days in vitro (6 DIV) and incubated with anti-ChAT, anti-HB9 and anti-βIIITubulin. To induce SMN overexpression or endogenous reduction of SMN or Gemin3, we generated lentiviral particles that were propagated in HEK293T cells. Human or mouse MNs were transduced with the lentiviral-based vectors after 3 hours in culture. Moreover, we analyzed Gemin3, IKKβ, and RelA protein levels in our models by Western Blot or immunofluorescence. We also analyzed IKKβ and RelA mRNA levels in SMA and control human MNs by quantitative RT-PCR.

Gemin3, IKKβ and RelA, were reduced in SMA MNs compared to control. Gemin3 knockdown reduced SMN, IKKβ and RelA protein level in cultured MNs, causing significant neurite degeneration. Furthermore, SMN overexpression increased Gemin3 protein in cultured MNs. However, SMN overexpression did not prevent neurite degeneration in Gemin3-knockdowned MNs.

These results indicated that Gemin3 reduction may contribute to MNs degeneration in SMA, providing new evidence to identify targets for the development of complementary therapies.

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P169

SMN protein level as a driver of selective motor neuron vulnerability

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Spinal Muscular Atrophy (SMA) is a motor neuron disease (MND) characterized by the loss of SMN protein due to homozygous deletion of the *SMN1* gene. Severe reduction of SMN protein leads to downstream effects that culminate in motor neuron (MN) degeneration, paralysis, and premature death. As one of the most common genetic neurodegenerative diseases, intense research has resulted in 3 FDA approved therapies which improve functional SMN protein production, thereby increasing patient mobility and longevity. Despite these breakthroughs, these treatments do not constitute a cure. Interestingly, MN degeneration patterns are not consistent across MN subtypes (i.e. some persist throughout a patient's life while others are lost early in disease progression). Understanding why some MN populations are resistant to degeneration may be key to identifying pathways that confer this resistance allowing for more efficacious combinatorial therapies that can protect vulnerable MN populations before they are lost. Interestingly, *in-vitro* studies have identified that MN populations—with SMA or healthy genetic backgrounds—exhibit heterogeneous SMN protein levels where higher expression correlates with improved MN survival. Additionally, cytosolic calcium modulation has been identified as a regulator of *SMN* gene expression, while vulnerable SMA MNs have been shown to display hyperexcitable properties. Therefore, we hypothesize that SMN protein heterogeneity result in a range of MN vulnerability or resistance, and that it is potentially regulated by intrinsic MN activity and downstream calcium homeostasis.

A direct relationship between SMN expression and MN survival was explored by employing live, longitudinal imaging and single cell quantification of iPSC-derived MNs where the native *SMN2* locus was converted to *SMN1* and C-terminally tagged with a fluorescent reporter to track SMN protein expression live. Calcium imaging of MNs was performed to correlate SMN expression with intrinsic activity properties of MNs on a single cell level.

On a single cell level, a MN's survival is directly correlated to SMN protein expression thereby establishing SMN protein heterogeneity as a mechanism that may intrinsically poise MNs to be vulnerable (low SMN) or resistant (high SMN). Furthermore, calcium imaging of a single MN population revealed that the lowest, and most vulnerable, SMN expressing ones are more active than their high expressing counterparts.

Our results lead to a new understanding of the importance of MN diversity in the progression of SMA. These results establish SMN protein heterogeneity as a driver of selective neurovulnerability and that intrinsic MN activity is correlated with SMN protein expression. Together this data provides the basis to identify targetable mechanisms that may regulate the heterogeneous expression of SMN protein and therefore the selective death of MNs in SMA.

P170

The SMN protein interacts with actin and is involved in the regulation of actin cytoskeleton dynamics

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Reduced SMN protein levels result in impaired actin cytoskeleton dynamics causing defects in neurite outgrowth, axonal pathfinding, and formation of functional synapses in neurons. This leads to the degeneration of α-motoneurons in the brain stem and spinal cord. Recently, we have demonstrated the implication of SMN and the neuronal actin- and SMN-binding protein profilin2a in fine-tuning the dynamic properties of the actin cytoskeleton. However, the role of a direct SMN-actin interaction in the regulation of actin dynamics with respect to its capability to regulate actin polymerization and remodelling is still elusive.

We studied the interaction of recombinant wildtype SMN and SMN-truncation variants with purified monomeric (G-) and filamentous (F-) actin using *in vitro* pulldown assays to identify the binding site for actin in SMN. Using Microscale Thermophoresis (MST) in combination with total internal reflection fluorescence microscopy (TIRFM) we characterized the molecular interaction between SMN, profilin2a and G- and F-actin, respectively, and analyzed the influence of SMN on actin polymerization.

Our MST and *in vitro* pulldown assays reveal a direct interaction of SMN with G- and F-actin. Furthermore, we identified a distinct region at the N-terminus of SMN as binding site for G- and F-actin. Our TIRF experiments provide evidence that SMN directly affects actin polymerization independent of profilin2a.

Our results suggest that SMN can function as direct regulator of actin dynamics. Thus, unraveling molecular details of the SMN-actin interaction and accompanying regulatory features of SMN on actin dynamics is essential for elucidating the pathological mechanism underlying spinal muscular atrophy (SMA), providing novel strategies for the development of alternative and combinatory SMA-treatments.

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Splicing regulation of Reticulon is involved in preventing neurodegeneration in a *C. elegans* model of SMA

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An efficient splicing of mRNA is required in all cells, but neurons seem to be more vulnerable to splicing perturbations. In fact, numerous neurodegenerative diseases are caused by splicing defects, including SMA. We previously demonstrated that genes differentially expressed or spliced in induced pluripotent cell-derived motor neurons (iPS-MNs) from SMA patients are enriched in the RNA motif 7. This motif is specifically recognized by hnRNP Q, a spliceosomal component physically and genetically interacting with SMN.

Using different *C. elegans* SMA models we identified Reticulon as a new genetic interactor of *smn-1,* the *Smn* homolog in this animal model. Neurodegeneration was assessed using fluorescent microscopy to analyze MNs survival in living animals. Moreover, we analyzed the alternative splicing pattern of Reticulon using semiquantitative PCR and transgenic animals that allows to visualize *in vivo* the alternative splicing of specific exons. By using molecular biology (qRT PCR) we evaluated the expression levels of Reticulon genes in a severe *C. elegans* SMA model, in SMA mice and iPS-MNs from SMA patient.

We demonstrated that *hrpr-1,* the *hnRNP Q* homolog in *C. elegans*, is involved in MNs survival similarly to *smn-1*. We confirmed *hrpr-1*/*smn-1* genetic interaction by nonallelic non-complementation and demonstrated that they exert their neuroprotective function specifically in MNs. In fact, *hrpr-1* overexpression in MNs rescues *smn-1* related neurodegeneration. Interestingly, comparing *hrpr-1* known targets in *C. elegans* and the alternatively spliced genes identified in iPS-MNs from SMA patients, we identified a new possible downstream target of the pathway, *ret-1*. *ret-1* is the only homolog in *C. elegans* of Reticulon genes, a family of transmembrane proteins involved in vesicle recycling and formation, and in neurite outgrowth. We identified for the first time a role of *Reticulon* in SMA, since we observed alteration in its transcript levels in a severe *C. elegans SMA* model, in SMA mice and in iPS-MNs from SMA patients. Moreover, we demonstrated that *ret-1* splicing pattern is altered when *smn-1* is depleted, and that *ret-1* is mediating the *hrpr-1* rescue of *smn-1*-related neurodegeneration. Finally, we demonstrated that *hrpr-1* and *smn-1* work together to guarantee the correct splicing of *ret-1* exon 5, and that exon 5 inclusion in *ret-1* transcript is necessary to prevent *smn-1* related neurodegeneration.

We identified for the first time a neuroprotective role of Reticulon in SMA and we demonstrated that its correct splicing is synergistically regulated by *hrpr-1* and *smn-1*.

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Criteria for identification and accurate quantification of spinal motor neurons in healthy and disease mouse models

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Motor neuron (MN) death is the hallmark of the MN diseases spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS). Quantification of MN loss in mouse models is an important readout for disease progression and therapeutic assessment. The large variability in MN death reported by different groups, even within identical mouse models, may depend on different technical approaches to label MNs as well as investigating distinct areas of the spinal cord with differential vulnerability.

MNs of selected segments of lumbar spinal cords of BL6 mice were labelled via ventral-root backfills and afterwards immuno-stained for choline acetyltransferase (ChAT). Whole lumbar spinal cords were cleared and imaged via confocal microscopy. Furthermore, selected spinal segments of SMNΔ7, SOD1-G93A and control animals were cut at a vibratome, processed for ChAT immunoreactivity, and imaged via confocal microscopy. Additionally, spinal sections of BL6 mice were stained for ChAT, SMI-32 and Nissl or ChAT and Hb9 and imaged.

Here, we established several morphological criteria to ensure consistent quantification of MNs. First, we describe a ventral-side-up spinal cord dissection, allowing segment specific MN isolation and counting. In combination with *ex vivo* ventral-root back fills and immunohistochemistry, we conclude that ChAT and HB9 are a reliable set of markers for MN identification combined with position in ventral horn. In contrast, Nissl and SMI-32 immunoreactivity are not selective and therefore, not suitable for MN number quantification. Second, ventral-root back fills of MNs within select lumbar segments with different fluorochromes, combined with tissue clearing and ChAT immunoreactivity showed that different spinal segments contain different numbers of MNs. Third, MNs marked with HB9 in select spinal segments were counted by an automated open-source counting plug-in for FIJI ImageJ software to provide an unbiased quantification of MNs. Fourth, comparison of MNs within the lumbar enlargement of SMA mice revealed a progressive degree of MN loss from L1 to L6. Interestingly, while a severe SMA mouse model exhibits selective MN death restricted to specific spinal segments, MN loss was evident throughout the entire axis of the spinal cord in the ALS mouse model SOD1-G93A.

Our detailed procedural account demonstrates that a select set of criteria is required for the valid identification of motor neurons and their accurate quantification in normal and disease mouse models. Furthermore, our results can be used as a reference for future studies requiring accurate assessment of MN counts as part of therapeutic assessment.

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Inhibition of JNK and GSK3 signaling pathway promotes spinal motor neurons survival and muscle improvement in human *in vitro* SMA co-culture system

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Spinal muscular atrophy is one of the most common genetic form of motoneuron diseases in childhood. Due to mutations in the SMN1 gene, SMA is characterized by an insufficient amount of SMN (survival motor neuron) protein, resulting in denervation, skeletal muscle atrophy and death. In the last decade, three treatments increasing SMN protein levels have been approved by the US Food and Drug Administration and result in impressive benefits for the patients. However, if those treatments are administrated at an advanced stage, their efficiency is highly reduced. For those patients with advanced symptoms, an important step forward would be to develop therapeutic approaches targeting pathways that are independent of SMN. However, predictive and functional SMA *in vitro* models for drug screening are still lacking. In this context, we developed a human SMA *in vitro* co-culture system between pluripotent stem cells-derived spinal motoneurons and their muscular targets adaptable to screening format in 384 well plates. These inter-cellular systems reproduced the specific mortality of SMA spinal motoneurons as well as their pre-synaptic defects. To evaluate to suitability of our system for drug screening, we performed a target-agnostic based drug screening revealing the potential of JNK3 and GSK3 inhibitors to normalize the cellular phenotypes associated to SMA independently of SMN expression.

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Stathmin-2 as a novel *SMN*-independent therapeutic target in *in vitro* and *in vivo* SMA models

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The therapies currently approved for Spinal Muscular Atrophy (SMA), which focus on Survival Motor Neuron (SMN) protein restoration via mRNA splicing modifier or gene replacement, surely represent a real revolution in the therapeutic approach to this disease. Nevertheless, critical issues such as possible long-term effects, extremely limited therapeutic window of the approved drugs and inter-individual response variability depending on disease severity still exist. Therefore, a possible approach to overcome these limits could be the development of complementary therapies acting on novel *SMN*-independent targets. Recent studies demonstrated that the neuronal microtubule regulator Stathmin-2 (STMN2) is dysregulated in neurodegenerative disorders. Axonal defects in Amyotrophic Lateral Sclerosis (ALS) are rescued by STMN2 overexpression *in vitro* and via its molecular misprocessing correction *in vivo*. These data paved the way for the launch of a clinical trial in ALS based on the *STMN2* modulation by antisense oligonucleotide. A possible role of STMN2 in SMA was hypothesized by our group based on evidence of STMN2 dysregulation in SMA MNs compared to control ones.

The aim of this study was to investigate the possible therapeutic application of STMN2 modulation in *in vitro* and *in vivo* SMA models, unraveling its possible interaction with SMN.

The overexpression of STMN2 by lentiviral particles ameliorated the typical pathological features in iPSC-derived SMA MNs, in terms of axonal integrity/length and dendritic complexity, in line with known STMN2 role in the microtubule compartment. Notably, similar amelioration was also obtained by administering SP600125 which reduces the phosphorylation and degradation of STMN2, inhibiting JNK1 kinase. Similarly, in the severe pre-symptomatic SMAΔ7 mouse model, the treatment with AAV9 encoding *Stmn2* was able to improve the survival rate and ameliorated motor phenotype and histologic features mainly related to muscular compartment and neuromuscular junctions. Interestingly, SMN modulation performed *in vitro* and *in vivo* models did not influence STMN2 expression, suggesting SMN-independence from STMN2 protein dysregulation.

Taken together, our data provided evidence that STMN2 may act as an SMN-independent protective modifier of SMA, paving the way for advancement in understanding the pathological mechanism at the basis of this disease. Positive effects were present in both *in vitro* and *in vivo* SMA models, identifying *STMN2* as a possible modifier gene suitable for gene therapy or pharmacological modulation in SMA and other MN diseases.

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Dysregulation of calcium dynamics in different in vitro models of spinal muscular atrophy

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Transient oscillations of intracellular Ca^{2+} play a crucial role in neuronal cell physiology by modulating processes such as synaptic transmission, protein phosphorylation and gene transcription. To investigate whether motoneuron (MN) pathology in spinal muscular atrophy (SMA) is accompanied by a dysregulation in Ca^{2+} homeostasis, we applied Ca^{2+} imaging fluorescent procedures to assess changes in neural activity and Ca^{2+} dynamics in SMA pathology.

hiPSC from control and SMA type II patients were differentiated to MNs and used after 19-23 days *in vitro* (div). Additionally, primary cultures of purified MNs from SMNΔ7 mice (control and SMA) at embryonic day 13 were also used after 9-10 div. For intracellular Ca^{2+} measurements, cells were incubated with FURA-2 AM. After recording basal activity, Ca^{2+} mobilization was pharmacologically analyzed. Different drugs were applied such as glutamate receptors agonists (kainate [KA] and N-methyl-D-aspartate [NMDA]) and endoplasmic reticulum (ER) Ca^{2+} mobilizing drugs (caffeine and thapsigargin).

hiPSC-derived MNs showed no differences in basal intracellular Ca^{2+} levels when comparing cells from control and SMA type II patients. By contrast, in MN primary cultures from the SMNΔ7 mouse line, SMA MNs exhibited slightly lower basal Ca^{2+} levels compared to control. In both types of cultures, SMA MNs showed more frequently rhythmic patterns of spontaneous activity compared to control MNs. However, depending on the type of culture used for analysis, there were significant differences in the proportion of MNs that responded to different stimuli: purified mouse MNs showed a higher response rate, which was more homogeneous than that found in hiPSC-derived MNs. hiPSC-derived MNs from SMA type II patients showed a reduction in the KA-induced Ca^{2+} transients amplitude. However, this result was no observed in mouse SMA MNs. After NMDA stimulation, no changes in Ca^{2+} dynamics were observed in any type of culture. ER Ca^{2+} mobilization experiments performed on KCl depolarized hiPSC-derived MNs showed a significant increase in Ca^{2+} release following stimulation with either caffeine or thapsigargin.

Our results indicate that decreased expression of survival motor neuron (SMN) in isolated MNs, causes important changes in intracellular Ca^{2+} dynamics. The impact of these alterations on MN excitability and on the neuronal degeneration described in SMA should be further analyzed. In addition, these two in vitro models can be considered as complementary paradigms to examine the alterations in Ca^{2+} homeostasis and to evaluate the action of pharmacological agents.

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Alterations of microtubule plus end tracking protein $p150^{Glued}$ in an in vitro Spinal muscular atrophy model

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Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disease, which is caused by *Survival of motor neuron 1* (*SMN1*) gene mutations. SMN protein deficiency results in impairments of cytoskeleton including microtubules due to abnormal levels of proteins regulating microtubule dynamics. Previously, we showed a downregulation in microtubule plus end-binding 3 (EB3) protein and increased comet numbers in SMN depleted motor neuron-like cells. EB3 is a hub protein, which creates a molecular platform for binding of other plus-end tracking proteins (+TIP) on growing microtubule ends. Here, we hypothesized that dysregulations in EB3 could have an impact on other +TIPs. In this study, we created a protein interaction network of SMN with microtubule associated proteins (MAPs), including +TIPs using bioinformatic tools, and analyzed microtubule plus end tracking $p150^{Glued}$ and CLIP170 proteins in an in vitro SMA model.

Microtubule-associated protein lists were obtained from databases, and high-confidence direct and indirect interactions between SMN and MAPs were collected to create a network. Among all, levels of EB3-interacting proteins, namely, $p150^{Glued}$ and CLIP170 were analyzed by Western blot using SMN knock down motor neuron-like NSC34 cells. Immunofluorescence stainings and confocal imagings were performed for quantitative image analysis of $p150^{Glued}$ and CLIP170 comet-like structures by Image J program.

Candidate MAPs, having potential to functionally interact with SMN were identified via bioinformatic analysis. In SMN-depleted cells, significant upregulation was found in $p150^{Glued}$but not, CLIP170 protein level compared to controls. Quantitative microscopic analysis showed a significant increase in the number of comet-like structures, formed by $p150^{Glued}$ at both the proximal and distal part of neurites in SMN knock down cells.

Our results indicate that both level and distribution of $p150^{Glued}$ protein were impaired in SMN deficiency. $p150^{Glued}$ is the major subunit of dynactin, therefore, in addition to microtubule dynamics, dysregulations could also affect retrograde transport. Our findings pointing the contributions of motor proteins in SMA pathomechanisms and have been investigating in ongoing studies.

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Exploring human neuromuscular junction functionality in vitro: A custom micro electrode array (mea) approach for investigating Spinal Muscular Atrophy (SMA)

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Neurodegenerative diseases represent a challenge in the field of medicine and neuroscience. These disorders encompass a diverse group of conditions characterized by the progressive degeneration of neurons within the central nervous system. The emergence of pluripotent stem cells allows scientists to create patient-specific cellular models, in order to study the cellular and molecular mechanisms underlying various diseases, including neurodegenerative disorders. On the other hand, NMJ models are essential for understanding the complex interplay between neurons and myocytes at the neuromuscular junction how dysfunction in this critical communication can lead to neuromuscular diseases.

In this context, we have developed on-chip devices that allow us to grow hNMJs on specific electrode patterns. This setup enables us to stimulate pre-synaptic axons and record post-synaptic muscle activity, including action potentials. Our platform successfully replicates mature and functional in vitro hNMJs, as evidenced by the ability to trigger recordable extracellular muscle action potentials through electrical activation of motor neurons (MNs).
This study offers a physiologically relevant model for mimicking hNMJs and aims to investigate the distinct effects of motor neurons and myotubes on NMJ defects observed in patients with spinal muscular atrophy (SMA).

Preliminary data indicates reduced overall electrical activity in SMA hNMJs compared to healthy ones. In mixed hNMJs, initial results suggest that healthy muscles may positively influence SMA MNs electrical activity, while SMA muscles appear to impair healthy MNs electrical activity.

Further data is required to fully understand the impact of both cell types on hNMJ functionality. However, this innovative tool shows promise for compartment-specific drug screening, allowing us to assess drug effects not only on the treated cell type but also on NMJ functionality. Additionally, this on-chip device holds potential for characterizing NMJs and their disruptions in other neurodegenerative diseases.

P178

Neurotransmission and motor performance in one-year-old SMA and control mice expressing the chaperone variant Hspa8^{G470R}

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Spinal muscular atrophy (SMA) is a severe inherited neuromuscular disorder caused by the loss or mutation of the *SMN1* gene, resulting in a decrease in survival motor neuron (SMN) protein level and severe synaptic dysfunction at the neuromuscular junction (NMJ). SMN has been shown to associate with Hspa8, a constitutive molecular chaperone participating in proteostasis. Hspa8 promotes the folding and degradation of proteins and is highly expressed in neurons and at the NMJ. The expression of the Hspa8 genetic variant G470R (Hspa8^{G470R}) rescues most of the pathogenic phenotype of SMA mice (Kim et al. 2023). Hspa8^{G470R} has an enhanced affinity for synaptic co-chaperone proteins compared to wild type Hspa8, and prevents SNARE complex assembly level reduction in SMA mice in early postnatal and young adults.

We studied whether the functional rescue of Hspa8^{G470R} persists in one-year-old SMA mice by electrophysiology and motor tests.

Our results reveal that Hspa8^{G470R} efficiently maintains neurotransmission within normal levels in SMA mice at the TVA muscle, one of the most affected in the disease. Motor performance is normal till six months of age in adult SMA Hspa8^{G470R} mice but progressively decreases later. Interestingly, quantal content in control mice expressing the genetic variant is significantly enhanced (about 30%) compared with control mice expressing the wild-type form of the protein.

Our findings suggest that Hspa8^{G470R} specifically and efficiently prevents NMJ synaptic dysfunction through the lifetime of SMA mice and improves synaptic efficacy in control mice.

P179

A novel pathomechanism for spinal muscular atrophy based on SMN-cdc42 interaction regulating macropinocytosis and BMPR2

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Spinal muscular atrophy (SMA) is a childhood disease caused by insufficient levels of SMN protein and primarily affects motor neurons. An early pathologic event is the degeneration of neuromuscular synapses. Since BMP receptors (BMPRs) are critical regulators of synapse maintenance and downregulated by macropinocytosis, we investigated whether low levels of SMN protein affected macropinocytosis and BMPR levels.

A fluorescence-based, quantitative macropinocytosis assay was established for adherent cells using FITC-Dextran 70 as a macropinocytosis tracer and FACS-based quantification. A panel of inhibitors, size exclusion chromatography and selective activation procedures were employed to characterize constitutive and induced macropinocytosis pathways in in motor neuron-like NSC34 cells. SMN levels in NSC34 were titered using different SMN siRNAs. Alternatively, primary motor neurons from Taiwanese SMA model mice were enriched by density gradient centrifugation of spinal cord cells obtained from 12.5 d old embryos. In some experiments, primary fibroblasts from an SMA type 0 patient were used as cell source. For proximity analysis in live NSC34 cells, a Bio-ID screen was performed and protein-protein interactions confirmed in-vitro by co-immunoprecipitation and functional assays. Levels of BMPR2 and other proteins were quantified by Western blotting.

We describe a novel constitutive macropinocytosis pathway that is upregulated in motor neuron-like NSC34 cells upon knockdown of SMN, in primary motor neurons from a severe mouse model of SMA and in fibroblasts from an SMA type 0 patient. Inhibition of the small GTPase cdc42 mirrored the effect of SMN knockdown on macropinocytosis suggesting that cdc42 might serve as a link between SMN and macropinocytosis. A proximity analysis revealed cdc42 as a high scoring potential SMN interactor. SMN could be co-immunoprecipitated with nucleotide-free cdc42 from lysates of NSC34 cells, suggesting that SMN acted as a GTP exchange factor (GEF) of cdc42. Upon knockdown of SMN, BMPR2 was severely decreased in NSC34 cells due to Bafilomycin-sensitive degradation in late endocytic organelles.

We propose a novel pathomechanism for SMA based on an SMN-cdc42 interaction that controls a constitutive macropinocytosis pathway shuttling a critical regulator of synapse maintenance to endosomes/lysosomes for degradation.

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Roche

Roche-sponsored satellite symposium
Van Rysselberghe Room, Ghent ICC, Ghent, Belgium
Thursday, 14 March 2024 | 13:10–14:10

Should all adults with SMA receive treatment? **An experts' debate**

Join us at our **satellite symposium** where we will explore the benefits of, and barriers to, adults receiving treatment for SMA

In the form of an exciting debate, our faculty speakers will argue their case on **whether all adults living with SMA can and should receive disease-modifying treatment**

Prof. Laurent Servais
Professor of Paediatric Neuromuscular Diseases,
MDUK Oxford Neuromuscular Centre,
Department of Paediatrics,
University of Oxford,
Oxford, UK

Prof. Blaž Koritnik
Institute of Clinical Neurophysiology,
University Medical Centre Ljubljana,
Ljubljana, Slovenia

Department of Neurology,
Faculty of Medicine,
University of Ljubljana,
Ljubljana, Slovenia

Ms. Simona Joveska
Social Services Advisor
for Vulnerable Groups,
Municipality of Bitola,
Bitola, North Macedonia

Programme

Living with SMA as an adult

Debate

Audience Q&A

Visit us at the Roche booth (**Booth #1**) to learn more about our commitment to SMA and speak with our representatives

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NOVARTIS

SMA JOURNEYS: PERSPECTIVES FROM CAREGIVERS AND CLINICIANS

Join us for a dynamic caregiver–clinician discussion aiming to inspire your clinical practice!

Friday 15 March 2024 | 13:10–14:10 CET

Theo Van Rysselberghe room,
Gent International Convention Center

The spinal muscular atrophy (SMA) journey is an individual experience for all patients and their families.

In this symposium, caregivers will take to the floor alongside experienced clinicians to discuss their SMA journeys.

How can we improve every unique patient experience and optimise the SMA pathway?

Meet the faculty | Clinicians

Prof. Liesbeth De Waele | University Hospitals Leuven, Leuven, Belgium | **Chair**

Prof. Ludo van der Pol | University Medical Center Utrecht, Utrecht, Netherlands | **Speaker**

Prof. Arnaud Vanlander | Ghent University Hospital, Ghent, Belgium | **Speaker**

Meet the faculty | Caregivers

Caregivers of children with SMA treated by **Prof. Liesbeth De Waele** and **Prof. Arnaud Vanlander** will join the clinicians on stage to discuss their SMA journeys.

Agenda

Time	Session
13:10	Welcome and introductions
13:20	Healthcare professional and caregiver perspectives on communication, joint decision-making and multidisciplinary care
14:00	Audience Q&A, summary and close

We are looking forward to welcoming you in Ghent at SMA Europe 2024!

A non-promotional symposium organised and funded by Novartis Gene Therapies
MED-CON-UNB-2024-00003-EU | Date of preparation: February 2024

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