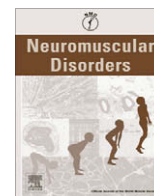




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Workshop report

Developing standard procedures for murine and canine efficacy studies of DMD therapeutics: report of two expert workshops on “Pre-clinical testing for Duchenne dystrophy”: Washington DC, October 27th–28th 2007 and Zürich, June 30th–July 1st 2008

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1. Introduction

Despite extensive research and pre-clinical testing on animal models, there is no consensus regarding the most appropriate design of pre-clinical trials, and most appropriate clinical, functional, histological, imaging and biochemical endpoints. Comparison of results obtained in different laboratories and prioritization of treatment options prior to clinical trials is therefore hindered. The Network of Excellence TREAT-NMD (Translational Research in Europe for the Assessment and Treatment of Neuromuscular Diseases) and Washington DC-Wellstone muscular dystrophy center have taken a lead to draft guidelines for most appropriate methods and endpoints for *mdx* and GRMD canine models.

The Network of Excellence TREAT-NMD addresses the fragmentation currently hindering translational research in the development of new therapeutic strategies for rare neuromuscular diseases. One aspect of the project (led by Santhera Pharmaceuticals Ltd.), is the acceleration of the pre-clinical phase of new treatment development, a critical step towards the final therapy's evaluation in humans. It aims at identifying and selecting (1) appropriate mammalian models for DMD, (2) appropriate readout parameters for efficacy studies of new potential treatments and (3) appropriate protocols (“standard operating procedures”, SOPs) to evaluate such endpoints. Similar goals are also pursued by National Institutes of Health (NIH) sponsored Washington DC-Wellstone Muscular Dystrophy Cooperative Research Network in the United States of America. A consensus paper on the choice of the most appropriate animal models was published this year [1] and details of methodology and endpoints that would benefit larger muscular dystrophy community are also discussed in recent publications [2,3]. Therefore the Wellstone Muscular Dystrophy Center at the Children's National Medical Center in Washington, DC and TREAT-NMD Neuromuscular Network collaborated on this project

and organized two expert workshops on the standardization of protocols for the most common endpoints in the recommended models (mice and dogs) to evaluate Duchenne Muscular Dystrophy therapeutics. Outcome of the workshops is a collection of standardized operating procedures (SOPs) that are currently available on the website <http://www.treat-nmd.eu/research/preclinical/SOPs/> and will also be posted on NIH Wellstone Portal (http://www.wellstonemdccenters.nih.gov/shared_resources.htm) with links to individual Wellstone Cooperative Research Centers in the United States.

2. Wellstone Center workshop “Pre-clinical Testing for Duchenne Dystrophy: Endpoints in the *mdx* Mouse” Washington DC, USA, October 27th–28th 2007

2.1. Animal models, methods and variability in pre-clinical evaluations

The first session on day one started with a welcome note and brief introduction of goals and objectives of the workshop by the organizer and director of the workshop, Kanneboyina Nagaraju. First speaker, Eric Hoffman outlined currently available animal models (e.g., dogs, cats, zebra fish and *Caenorhabditis elegans*), their advantages and disadvantages for pre-clinical studies. He also described recent global gene expression data from his laboratory supporting the hypothesis that inappropriate cross-talk between various pathways results in failed muscle cell regeneration and noted that strategies to correct these cross-talks may help to reduce disease progression in DMD. Kanneboyina Nagaraju described the state-of-the-art pre-clinical phenotyping and drug testing facility for muscular dystrophies that he has set-up at the Children's National Medical Center (CNMC). He described various behavioral (rota-rod, open field activity monitors), functional (grip strength, *in vitro* force contractions on isolated muscle), imaging (echocardiography, optical Imaging and MRI), histological (H&E, fibrosis estimation by sirius red), biochemical (serum creatine kinase) and molecular evaluations (Western blotting, immunohistochemistry

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and gene expression profiling) techniques that are currently being used to evaluate therapeutic efficacy in *mdx* mouse model at the CNMC facility. He emphasized the need to develop open shared data bases for pre-clinical studies in the *mdx* mice and importance of developing uniform guidelines for all the pre-clinical evaluation methods of muscular dystrophy mouse models. Annamaria De Luca described the advantages and effect of exercise regimen, and challenges associated with the exercise (variability, active avoidance) in the *mdx* mouse model. She pointed out that chronic exercise on treadmill is useful to exacerbate the skeletal muscle pathology detected using functional, histological and biochemical parameters in *mdx* mice. She described merits, demerits and methods of interpretation for several techniques (grip strength, electrophysiology, microspectrofluorimetry, isometric contractions histology and biochemical techniques) that she routinely uses to evaluate compounds in the *mdx* mouse model of dystrophy. Miranda Grounds outlined strategies to reduce the biological variation and emphasized the need (a) to develop a core set of methods that everyone can use to evaluate drug efficacy and (b) to develop a standard operating procedures and a unified scale to measure therapeutic efficacy in the *mdx* mouse model.

The second session of the morning was focused on the early signaling events in dystrophic mouse muscle, evaluation of respiratory dysfunction and use of multi-parametric scale evaluation of pre-clinical efficacy for *mdx* mice. Urs Ruegg discussed methods to evaluate calcium dysregulation, proteolytic activation and metabolic impairments in dystrophic skeletal muscle. Tejvir Khurana described respiratory dysfunction, effect of hypoxia on exercise, changes in blood gas levels in young and old *mdx* mice and use of whole-body plethysmography to monitor respiratory dysfunction in the *mdx* mouse model. He noted that the evaluation of respiratory function should be included as an endpoint to monitor therapeutic efficacy in the *mdx* model. Sasha Bogdanovich discussed advantages of using a single numerical value to assess therapeutic efficacy and proposed a multi-parametric scale (body weight, muscle mass, force (specific and absolute), eccentric contraction, creatine kinase and central nucleation in muscle fibers) for cross-comparing different pre-clinical studies and prioritizing drug development for muscular dystrophy therapy in the *mdx* mouse model.

2.2. Muscle function and histology readouts in *mdx*, toxicology evaluations

The first afternoon session was focused on muscle physiology methods, *in vivo* and *in vitro* muscle function, and whole body tension. Elisabeth Barton described various muscle physiology parameters and noted that evaluation methods should depend on the type and nature of therapy (gene replacement, calcium handling, pro-growth strategies). She indicated that certain measures are more affected by the therapy than others and emphasized the need for developing a decision making tree. She mentioned that the extent of damage of single muscles (diaphragm, *extensor digitorum longus* and *soleus*) may vary at different age groups in *mdx* mice and outlined some of the challenges of performing muscle physiology experiments. George Carlson described two noninvasive methods (whole body tension (WBT) and the hang impulse) to assess muscle weakness in the *mdx* mouse model. He noted that WBT determinations are positively correlated with *gastrocnemius* twitch and tetanic tension and that the physical impulse associated with the hang test measures the ability to produce sustained tension in the limb musculature. Gordon Lynch gave a detailed account and highlighted advantages and disadvantages of various *in vitro*, *in vivo* and *in situ* techniques available to measure muscle function in the *mdx* mouse model. He noted that accurate endpoint

measurements provide critical information about therapeutic efficacy and that the use of more than one technique provides complementary information at different levels (whole muscle or cell/fiber).

The second session of the afternoon discussed quantitative measure of histology behavioral and motor function methods, and pre-clinical toxicology evaluations. Markus Ruegg briefly outlined regulatory requirements and specific objectives of TREAT-NMD (Activity 07) and discussed quantitative assessment of histology in *mdx* mice. He noted that variance coefficient of minimal Feret's diameter is a sensitive measure of the pathological change. He also pointed that the number of centralized nuclei and determination of the content of hydroxyproline in muscle are reliable measures of drug efficacy in *mdx* mouse model. He noted that there should be concerted efforts of groups (TREAT-NMD and other researches) to reach a consensus on (a) disease-relevant and predictive animal model(s) and (b) appropriate methods to test efficacy of treatment. Olivier Dorchies discussed *in vivo* behavioral (wire hang test) and motor (isometric force) functions. He also noted that muscle structure and function assessments are poorly correlated and that assessment of kyphosis reflects back muscle weakness in *mdx* mice. Gopala Krishna discussed various steps in toxicology and pre-clinical candidate drug evaluations including drug discovery & development process/timeline, pre-clinical study effort, FDA's Center for Drug Evaluation and Research guidance link, clinical study effort and regulatory considerations.

2.3. Cardiac function readouts and MRI in *mdx*, phenotype of the Golden Retriever dog model, pre-clinical and clinical FDA requirements

Chris Spurney outlined the method of cardiac imaging (high frequency echocardiography) that is currently used at CNMC pre-clinical mouse facility. He noted the statistical power of cardiac measurements and the usefulness to non-invasively evaluate cardiac function during therapeutic interventions. He also briefly outlined some of the strategies (e.g., isoproterenol administration) to improve the *mdx* model to study cardiac function. Andrew Hoey briefly described kyphosis index and effect on thoracic area in normal and *mdx* mice. He described various cardiac measurements ranging from *in vivo* (Echo, Millar Catheter, and ECG), *in vitro* (isolated heart, left atria and electrophysiology) and *ex vivo* on isolated cardiomyocytes (patch clamp, cell shortening and calcium measurements) and histology (fibrosis) to evaluate cardiac function in the *mdx* mouse model. Volker Straub described various cardiac evaluation methods (clinical symptoms, MRI, echocardiography and catheterization and histology) and reviewed published literature on this subject. He mentioned that there are several open questions (e.g., when to study cardiac function, which parameter should one assess and which range of cardiac volumes should one use for the assessment) that need to be discussed. He indicated that MRI evaluation has several strengths that include studying distribution of pathology, pathophysiology, monitoring of therapies, assessment of heart and diaphragm, and morphometry. However he noted that there is no bench mark data for *mdx* mice and no consensus on how to generate and analyze the data. He also briefly described the TREAT-NMD network and principal areas of its activity in Europe and around the world.

In the second session of the morning, Laxminarayan Hegde outlined the broad range of pre-clinical pharmacology evaluation methods and specifically discussed the drug discovery process, the factors influencing druggability of test agents, the role of surrogate endpoints and biomarkers, the predictive power of animal models and physiological endpoints relevant for pre-clinical drug evaluation using *mdx* mice. Joe Kornegay described the Golden Retriever Muscular Dystrophy (GRMD) dog model; how this model

mirrors human DMD and how the larger size of dogs is advantageous for studying pathogenesis and proof of concept therapeutic studies. He outlined several functional, biochemical, cardiac and respiratory measures that are used to evaluate the GRMD model. Lois M. Freed discussed non clinical FDA requirements, different types of investigational new drug applications such as standard investigational new drug (IND), exploratory IND (pharmacokinetics, pharmacodynamics, micro-dosing and mechanism of action) and botanical products/dietary supplements. She highlighted that pediatric indications may need juvenile animal studies (rodent, non-rodent), in which the age of animals at initiation should be appropriate for the study. Furthermore, developmental parameters (e.g., neurobehavioral, reproductive, skeletal) should be considered in evaluating drugs in young animals. Diana Escolar described various steps and planning of human clinical trials for DMD. She noted that proof-of-principle studies are not equivalent to therapeutic pre-clinical efficacy trials and that clinicians' input is important from the beginning of the drug development plan. She also outlined several regulatory check points, faster first in man clinical trials, human micro-dosing studies and their utility, exploratory INDs, derivation of starting clinical dose steps and planning early first in human trials. John Porter underlined the need of a rigorous experimental design and of solid efficacy data when studies on animal models are meant for translation to patients. Funding agencies require higher stringency than journal publications because of substantial resources invested. In this view, a consensus on endpoints and success criteria for the tested therapy would be undoubtedly useful.

2.4. Prioritization of endpoints and assignments of draft protocols

The afternoon discussion on prioritization of endpoints and assignments of draft methods was moderated by Kanneboyina Nagaraju and Annamaria De Luca, who initiated discussion on proof-of-concept versus pre-clinical studies, outlined the need to first develop guidelines for methods and identify primary and secondary endpoints to assess therapeutic efficacy in *mdx* mice. Participants agreed on the need to prioritize endpoint assays, design reliability studies and define improvement in these studies. Furthermore, it was agreed to draft step-by-step instructions for the methods discussed during the workshop. All protocols were posted for comments and discussion by the wider muscular dystrophy community on a password restricted TREAT-NMD website and finally discussed and finalized during the follow-up workshop in Zürich.

3. TREAT-NMD workshop “Pre-clinical Testing for Duchenne Dystrophy” Zürich, Switzerland, June 30th-July 1st 2008

3.1. Standard operating procedure (SOP), requirements, muscle function readouts in the *mdx* mouse

The morning session of the first day was opened by Markus Rüegg, who expressed gratitude to NIH, FED and TREAT-NMD for the funding support of the present and the past workshop on pre-clinical testing for Duchenne dystrophy. He then underlined the need for standardized operating procedures for *mdx* and GRMD as guidelines for efficacy studies of new treatments. He further explained how this necessity was felt by the scientific community and how it is now a goal of the European Network of Excellence TREAT-NMD to establish such protocols. Judith Dubach-Powell described key aspects of SOP writing and mentioned guidance documents. She underlined the need of implementing an approval procedure (by the author and one reviewer) and an updating procedure for each SOP and the need of a wide distribution to the

appropriate work areas. Finally, she proposed a template for SOP-writing. Pat Nolan described in his talk the scope and creation of the EMPReSS database for mouse phenotyping, the process used to validate the SOPs and the procedures to warrant the consistency of the methods between the different centers involved in the validation process.

The session dedicated to *mdx* protocols was opened by Kanneboyina Nagaraju who summarized the assignment of protocols and the creation of working groups as agreed at the Washington DC workshop. He referred to the existence of SOPs for pre-clinical work in Amyotrophic Lateral Sclerosis. He then described the protocol for open field digiscan and the special care that has to be taken to minimize changes in the environmental condition of mice cages. Annamaria De Luca presented the use of physical exercise in mice to assess the neuromuscular state of the animal, to worsen the phenotype for a better evaluation of treatments and to achieve beneficial effects. The swimming exercise protocol was generally regarded as too stressful for the animals and forced downhill running as too deleterious; and it was decided not to include these protocols in the SOPs. It was agreed that wheel and treadmill exercise that serve as functional readouts for a treatment should be described in SOPs separate from those that describe methods to worsen the phenotype. Elisabeth Barton described devices and procedures for muscle strength measurements and mentioned that the choice of muscles and age-related changes have to be considered in this protocol. She also observed that a standard set of protocols may not be able to address all potential therapies and that the applicability of one SOP depends on the treatment tested. Olivier Dorchie presented results on drug efficacy tests conducted using the hang wire test. Advantages of this method are ease of use and low costs, but disadvantages are the influence of body weight on the outcomes and the age limitation, since the test is not appropriate for mice younger than four weeks. He stated that this test is probably not yet in a stage to be included in SOPs. Jon Tinsley described the grip strength method as protocol used to measure muscle strength. He outlined the disadvantages of learning skills of mice, the high number of animals required to ensure reproducibility and the need of standardized diet to compensate body weight variations between mice. George Carlson presented a protocol for whole body tension measurement as very reliable and reproducible method. He tested reliability between laboratories and between trials and obtained good results. Inter-examiner variability seems to be higher; therefore he recommends that the same examiner assesses a treatment efficacy throughout a trial.

3.2. Cardiac and respiratory function, histology, biochemistry, electrophysiology readouts in *mdx*

The afternoon session of the first day started with Chris Spurney's presentation on echocardiography protocols. High frequency echocardiography was presented as superior as compared to other methods used to measure left ventricular function; moreover, it allows longitudinal studies and an expanded cardiac analysis. Disadvantages are the choice of the anesthetic gas and the close monitoring of experimental conditions. The P/V loops protocol used to assess cardiac contractility was presented by Paul Herijgers. He showed devices, methods and some published results; one disadvantage of this method is the minimum age limitation of twelve weeks because of heart size. Thomas Meier presented a protocol for quantitative histology assessment of muscle fibers that allows a reliable and sensitive way to determine muscle fiber diameter and centronucleation independently of the plane of section. Reference values for a number of muscles with this method are available. Miranda Grounds discussed H&E-based

morphological measurements. She pointed out that one important consideration using this method is the animal age. Moreover, she emphasized that acute necrosis may be a more useful readout in efficacy tests than chronic necrosis because differences are more evident in the first. The measurement of undamaged area, although difficult in adult muscles, may be convenient in cases of treatments that offer damage protection. She noted also that fibrosis assessment, useful in older mice, requires further markers (for instance hydroxyproline). The issue of gender needs to be standardized in this SOP. Urs Ruegg presented several methods for calcium measurements and proposed imaging methods (included Mn-quenching) and calcium-45 methods as appropriate for SOPs. The use of myotubes or myofibers has to be determined; adult fibers are considered more suitable for outcome measurements. Dominic Wells presented protocols related to creatine kinase and lactate dehydrogenase determination in blood. Advantages and disadvantages of these commonly used tests were described. Topics of discussion were blood sampling (because of country-related differences in the guidelines) and optimal blood storage before analysis. To address these issues it was agreed that dedicated SOPs would be useful. Philippe Gailly presented the patch-clamp technique for assessment of channel properties, open probability, channel occurrence etc., but considered these technique as not appropriate in efficacy studies. After some discussion it was agreed to nevertheless include electrophysiology in the SOP collection. Volker Straub described MRI as not appropriate for routine use because it is time consuming and very expensive and because a standardization of the protocol is not feasible. Instead, he proposed to develop an SOP for the preparation of animals to MRI measurements. Tejvir Khurana underlined the usefulness of measurements on respiratory function like tidal volume, respiratory rate, minute volume, and pointed at the problem of the large dead volume of commercial instruments showing some adaptations to overcome this inconvenience. He emphasized that an SOP on respiratory function could be difficult to validate at the present time but would nevertheless represent a good starting point for further developments. Finally, Jean-Marie Gillis proposed the use of a common score sheet that quantitates the recovery rate following a treatment, independently of the assay used, the treatment applied or the units of measurement. Such an approach would therefore facilitate comparison of results from different laboratories. It was generally accepted to include this score sheet in the collection of SOPs.

3.3. Golden Retriever Muscular Dystrophy (GRMD) readouts

The morning session of the second day was dedicated to GRMD protocols. Joe Kornegay presented a technique to measure tibiotarsal joint torque force that allows the definition of both flexion and extension. Devices and challenges were described. He also showed some published data and pointed at the paradoxical increase of flexor strength in GRMD muscles. A technique, adapted from mouse protocols, assessing the eccentric contraction force decrease was presented by Robert Grange. The device allows to measure eccentric and concentric force and the software for quantitation is available. Joe Kornegay presented two more protocols applied in dogs: the measurement of tibiotarsal joint angle and MRI. The first allows the quantitation of contractures, an important disease parameter occurring also in DMD patients. The method is very easy to use and data were presented that show how this parameter correlates with tibiotarsal extension force from the age of six months onwards. Although not yet widely used, this method could become a very useful tool for the comparison between laboratories. The system for MRI measurements was described together with preliminary data on fat deposition and atrophy of different dog muscles. In general, fat deposition in GRMD was found not to be as pronounced as in DMD patients. Jean-Laurent Thibaud also presented the NMR method for

quantitative and qualitative evaluation of muscles and even for visualization of drug distribution in the muscle. Finally, Stephane Blot presented protocols and data on echocardiography in dogs and a new way to measure changes in the dog's gait, adapted from a method already applied to horses. However, this protocol is still in the experimental phase and should not be included in the collection of SOPs for the time being.

4. Conclusions and further steps

Participants of both workshops agreed that standardizing of protocols for pre-clinical research is urgently needed, especially if one attempts to test efficacy of potential treatments. The effort of TREAT-NMD to propose appropriate animal models, readouts and protocols, based on the consensus of leaders in the field therefore comes at the right time and finds a well-prepared ground. Some concern was expressed regarding the wording "Standard Operating Procedures". However, it was made clear that a collection of SOPs is not meant to be mandatory for pre-clinical efficacy studies but to be a way to improve quality and make results between laboratories comparable.

It was agreed to form a working group of experts for each SOP to solve open questions and to finalize the SOPs. A list of draft SOPs was circulated and authors were defined in order not to have one author responsible for more than two SOPs. Workshop participants signed up in the working groups of interest. It was further decided to determine, from each working group, an official reviewer who will be co-responsible with the author for completing and updating the SOPs. A template for SOP writing was to be drafted to help focusing on key aspects of the SOP:

- easy to read and use
- step-by-step description of the procedure
- advantages and disadvantages
- limitations (for instance age and gender of animals; muscle analyzed etc.),
- criteria of interpretation and evaluation of results
- historical data

The template was worked out and distributed to SOP authors shortly after the workshop.

As already proposed in Washington, finalized SOPs were to be uploaded on the TREAT-NMD website; a web link was specifically created for this scope that allows download of protocols and contact to the author for questions and suggestions (<http://www.treat-nmd.eu/research/pre-clinical/SOPs/>); later on, SOPs will also be posted on NIH Wellstone Portal (http://www.wellstonecenters.nih.gov/shared_resources.htm). The SOPs will be updated annually by the author and reviewer, taking into consideration feedback and suggestions that they may have received from users. An administrative committee was nominated with the tasks to maintain SOPs, discuss the current status of SOPs, suggest new SOPs, involve further experts in the field and implement the use of SOPs. The administrative committee is composed of:

1. Volker Straub as TREAT-NMD representative
2. One representative of TREAT-NMD Activity 7
3. Annamaria De Luca
4. Miranda Grounds
5. Joe Kornegay
6. Kanneboyina Nagaraju

The dissemination of SOPs is further implemented by reports that were sent to participating associations shortly after the workshop, and by the present report.

5. Workshop participants

5.1. Wellstone Center/NIH Workshop, Washington DC, USA, October 27th–28th 2007

Elisabeth Barton (Philadelphia, USA), Sasha Bogdanovich (Philadelphia, USA), Kevin Campbell (Iowa City, USA), George Carlson (Kirksville, USA), Annamaria De Luca (Bari, Italy), Olivier Dorchies (Geneva, Switzerland), Diana Escolar (Washington DC, USA), Lois Freed (Bethesda, USA), Heather Gordish (Washington DC, USA), Miranda Grounds (Perth, Australia), Laxminarayan Hegde (California, USA), Andrew Hoey (Toowoomba, Australia), Eric Hoffman (Washington DC, USA), Tejvir Khurana (Philadelphia, USA), Joe Kornegay (Chapel Hill, USA), Gopala Krishna (Rockville, USA), Jennifer Lachey (Cambridge, USA), Gordon Lynch (Melbourne, Australia), Marielena McGuire (Congressional Directed Medical Research Programs, USA), Kanneboyina Nagaraju (Washington DC, USA), Glen Nuckolls (NIAMS, National Institute of Health, USA), John Porter (NINDS, National Institute of Health, USA), Bill Quirk (Foundation to Eradicate Duchenne, USA), Markus Rüegg (Basel, Switzerland), Urs Ruegg (Geneva, Switzerland), Chris Spurney (Washington, USA), Volker Straub (Newcastle, UK), George Vella (Charley's Fund, USA), Ljubisa Vitkovic (NICHD, National Institute of Health, USA), Joel Wood (Foundation to Eradicate Duchenne, USA).

5.2. TREAT-NMD/FED Workshop, Zürich, Switzerland, June 30th–July 1st 2008

Annemieke Aartsma-Rus (Leiden, The Netherlands), Elisabeth Barton (Philadelphia, USA), Didier Bertoldi (Paris, France), Stephane Blot (Maison Alfort, France), Heinrich Brinkmeier (Greifswald, Germany), Pierre Carlier (Paris, France), George Carlson (Kirksville, USA), Margot Coville (Nantes, France), Sabine De la Porte (Gif-sur-Yvette, France), Annamaria De Luca (Bari, Italy), Oliver Dorchies (Geneva, Switzerland), Judith Dubach-Powell (Liestal, Switzerland), Yves Fromes (Paris, France), Philippe Gailly (Leuven, Belgium), Jean-Marie Gillis (Leuven, Belgium), Robert Grange (Blacksburg, USA),

Miranda Grounds (Perth, Australia), Paul Herijgers (Leuven, Belgium), Tejvir Khurana (Philadelphia, USA), Joe Kornegay (Chapel Hill, USA), Jennifer Lachey (Cambridge, USA), Hervé Laouenan (Association Française contre les Myopathies, France), Thomas Meier (Liestal, Switzerland), Paul Muhrad (Muscular Dystrophy Association, USA), Kanneboyina Nagaraju (Washington DC, USA), Pat Nolan (Harwell, UK), Stefanie Possekel (Liestal, Switzerland), Jean-Marc Raymackers (Leuven, Belgium), Markus Rüegg (Basel, Switzerland), Urs Ruegg (Geneva, Switzerland), Chris Spurney (Washington DC, USA), Volker Straub (Newcastle, UK), Jean-Laurent Thibaud (Maison Alfort, France), Christian Thirion (München, Germany), Jon Tinsley (Abingdon, UK), Maaïke van Putten (Leiden, The Netherlands), Dominic Wells (London, UK), Raffaella Willmann (Basel, Switzerland).

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