



International Conference in Brussels, Belgium, 17-19 November 2009.

Bringing down the barriers

in translational medicine for inherited neuromuscular diseases.

On behalf of TREAT-NMD and NIH, who organized this meeting, I, **Günter Scheuerbrandt**, a biochemist from Germany, have written this report for all young and older people with neuromuscular diseases, and for their families and care givers. It contains summaries of the 27 presentations and of some of the discussions in between. I have tried to write this report in a way that will let you understand most of what was presented and discussed.

But I had to keep this text short, so I could not explain all scientific details. Thus, if you would like to really understand the different research approaches for a therapy of at least one of the diseases, Duchenne muscular dystrophy, you should read my research reports, which you can see on my internet pages and can download them from there as pdf files: www.duchenne-information.eu in English, Spanish, and German.

As in my other reports, I have written the names of the scientists at the beginning of the summaries without their

academic titles, many of them are professors and practically all have an MD or PhD degree.

The chairpersons of this conference were **Kate Bushby** of the European organization TREAT-NMD in Newcastle upon Tyne in the UK and **John Porter** of the American National Institutes of Health, NIH, in Bethesda near Washington DC in the USA. About 350 people from 26 countries, the research and clinical experts and representatives of our *neuromuscular community* attended the meeting to discuss the key issues that need to be addressed and the problems that need to be solved if new and promising therapies and treatments are to be rapidly *translated into the clinic*, that is, to be made available as fast as possible to you, the patients, wherever in the world you live.

The entire program together with the abstracts of the 129 posters shown at the meeting can be seen on the internet at the address: www.treat-nmd.eu/conference/TREAT-NMD%20Conference%20Abstract%20Book%202009.pdf.

Duchenne muscular dystrophy, the long march from the parts to the whole.

Opening keynote lecture by **Gertjan van Ommen**, Leiden University, the Netherlands.

During the first 120 years since *Guillaume Duchenne* described Duchenne muscular dystrophy in 1868 until the dystrophin gene and its protein dystrophin was found in 1986/87, not much happened that could help the patients. But it took another decade before a genetic technique for a therapy was proposed in the mid 90s: *Exon skipping* "to hide exons from the splicing mechanism". It should restore the reading frame in the messenger RNA, produce a shortened dystrophin protein and thus change the severe symptoms of Duchenne into the often much milder ones of Becker muscular dystrophy. The first grant applications for developing this technique were unsuccessful because exon skipping was considered "a nice party trick that would never work in reality".

But in 2000, the Dutch scientists could show that in cultured muscle cells from a Duchenne boy with a deletion of the exons 48-50, an antisense oligonucleotide (AO) against exon 51 really skipped that exon and produced a Becker dystrophin in the isolated muscle fibers. The first bottle of champagne was due, and then many more when the next steps on the way to an effective exon skipping therapy were successful, too.

The scientists soon realized that "Duchenne is a disease that wants to be cured" because without dystrophin, the

membranes of the muscles, but not those of other organs, develop holes and cracks. Thus the exon skipping drugs can get into the muscle fibers easily, they are *muscle-specific*. However, exon skipping will only be able to slow down the fast muscle degeneration of Duchenne to the much slower of Becker dystrophy. It will be *an effective therapy* but not a complete cure.

Now, four clinical trials with Duchenne boys, who need skipping of exon 51, were performed with positive results, two local ones on one single muscle for a *proof of concept*, and two systemic ones with the injections of the drugs into the blood stream to reach all muscles.

Skipping of one or more exons would help about 80% of all Duchenne patients. The largest group of patients, 13% of all, would need skipping of just the one exon 51. The large pharmaceutical company *GlaxoSmithKline* will make milestone payments of up to US\$ 680 million to the Dutch company *Prosensa* in Leiden for the development of exon-skipping drugs against exons 51 and 44 and two others to be decided after the next large systemic clinical study with 150 Duchenne boys has hopefully shown a significant clinical benefit of this genetic technique.

During the next years, exon skipping will be fully developed for a therapy of Duchenne dystrophy, but there are

a number of other diseases for which this genetic technique may be used to repair proteins, to change their location in the cells, or to remove disease-causing proteins entirely.

Dr. van Ommen closed his lecture by stressing how important international registers will be, “biobanks”, with

Making clinical trials in neuromuscular diseases a reality.

Cristina Csimma (Viridante Pharmaceuticals, Cambridge MA, USA) chair person of the *TREAT-NMD Advisory Committee for Therapeutics*, TACT, started the first session of the meeting with her presentation **Moving forward with TACT** explaining how this new committee, established by TREAT-NMD at the beginning of 2009, will help academic and industrial researchers, who are evaluating therapies for neuromuscular diseases. The TACT review and assessment is intended to help applicants prepare and perform clinical trials by taking into consideration the scientific potential as well as a realistic development path that facilitates potential for later marketing approval of their drug candidates. The reviews will be done in close partnership between scientists, clinicians, patient organizations, and with industrial and regulatory drug development input.

TACT includes 42 members with broad scientific and development expertise in neuromuscular diseases (NMD). Their names, specialties, and pictures can be seen on the internet at: www.treat-nmd.eu/about/governance/TACT/.

NMD researchers and clinicians from academia or industry, wherever they are, can ask TACT for an assessment of their research strategy as they start considering advancing a compound into clinical trials in order to obtain advice, that can help strengthen their program and prepare it for clinical trials implementation. This early advice will hopefully provide a more efficient path forward and avoid mistakes and duplications.

The assessment report of TACT will be objective and confidential advice without charge, mainly based on the research plan, the pre-clinical data and any available clinical data in other diseases. A summary of the TACT assessment will be posted on the TREAT-NMD website in order to provide information and transparency to the broader community. The researcher will receive the TACT assessment report within 6 weeks after the TACT meeting, which will base its review on the application completed in advance of the meeting (a 15-page form can be downloaded from the internet address above). The first drug review meeting of TACT will take place on 6 and 7 February 2010 in Rome to consider three applications for the indication Duchenne dystrophy: Losartan, Isosorbide, and Flavocoxid. Applications for the second review meeting on 5 and 6 June in Barcelona can be sent until 15 March 2010 to emma.heslop@ncl.ac.uk.

To present the second example of an early cooperation between selected experts, **Michael Shy** (Wayne State University, Detroit, USA), of the CMT Association CMTA (www.charcot-marie-tooth.org) spoke about **The STAR initiative to develop therapies for Charcot-Marie-Tooth disease (CMT)**.

standardized data of patients who have rare and not so rare diseases that are still untreatable. This would give scientists the opportunity to detect the molecular mechanism of a disease in all details so that a precisely targeted successful genetic technique could be developed for an effective therapy or even a complete cure.

CMT affects about 2.5 million people worldwide. It is not a life-threatening disease, but the patients lose slowly the normal use of their feet, legs, hands and arms as their muscles weaken because the nerves leading to them degenerate. About 50% of the patients have CMT1A, the most frequent of the more than 30 different genetic causes of CMT.

CMT1A is caused by a duplication in one of the genes on chromosome 17 that cause the overproduction of the *peripheral myelin protein 22*, PMP22 in the Schwann cells which produce myelin, the insulating matter that surrounds the nerve fibers, the axons. Too much PMP22 leads to the deterioration of these nerves and causes atrophy of the muscle tissue to which they are supposed to send the signals from the brain.

For a therapy of CMT, the amount of PMP22 must be reduced. To find a candidate drug that would do this, CMTA founded the STAR Initiative, the *Strategy to Accelerate Research*, with assistance from Dr. *John Porter* of the National Institute of Neurological Disorders and Stroke, NINDS. This initiative is a partnership between the patient organization CMTA and people from business, academia and government.

The first task was to develop an automated *high throughput screening technique* to find among a very great number of chemical compounds those few, called *hits*, that would show in cultures of isolated modified Schwann cells the desired properties of a possible CMT drug, namely the downregulation of the PMP22 protein. Based on the advice of the STAR experts and with the help of venture capital, several hundred thousand compounds are being tested at the NHI Genomic Center. Among them, 10 to 12 promising compounds have been identified to date, which will now be subjected to additional screens, then chemically optimized before they can become candidates for clinical tests with patients.

John Porter, the third presenter in this first session of the conference, said that both STAR and TACT show very clearly how the barriers at the research steps can be brought down to accelerate the translation of successful science “*from bench to bed*” for the benefit of the patients and their families.

This approach needs to be applied also to other diseases. At present, Myozyme[®] against Pompe disease is the only approved drug on the market for a neuromuscular disease. But 239 million dollars alone in 2008 have gone as NIH grants into NMD research. With the words “it is time that we learn from each other; if we are not partnering, nobody will get a drug” John Porter finished his talk.

Target and candidate identification.

In this session on the methods to find possible chemical substances which could be used for a therapy of neuro-muscular diseases, **John Babiak**, (PTC Therapeutics, South Plainfield NJ, USA) spoke about the **First step in the search for critical therapeutics**. PTC means “Post Transcription Control”, because the specialty of this company is to develop drugs which would repair the damaged genetic message of a gene for a heritable disease after it is transcribed, copied, from the DNA to the mRNA in the nucleus of a cell. The first step to find a chemical compound that could be used for repairing the mistake in the gene, its mutation, is a laboratory technique called *high throughput screening*.

Many thousands of different small-molecular chemicals, which can be bought from specialized companies, are analyzed in highly automated laboratory instruments to determine whether they might have a positive effect on a disease process. A valid assay must be available that would give a measurable signal in a test system, which would be different for different diseases. To find a drug for Duchenne dystrophy, e.g., such an assay must indicate the appearance of dystrophin in precursors of muscle cells like myotubes which can be measured in a test system using the light-producing enzyme luciferase from fireflies.

But it is not necessary that the exact molecular mechanism, the pharmacokinetics, is known of how the disease symptoms are being caused by a gene mutation or some other process. *The screening is phenotypic, independent of a target.*

Those compounds that give a positive result in the screening procedure are called *hits*. In a long process involving many chemists and lasting many years, the most promising hits are optimized until a few them can become drug candidates that show the desired activities *in vitro*, in cell cultures, and *in vivo*, in living animals which that are a valid model of the human disease under study. The candidates must be non-toxic, easy to deliver, and easy to prepare in large quantities.

PTC has used high throughput screening for the identification and optimization of drug candidates for the suppression of nonsense mutations that cause a premature stop codon in about 15% of Duchenne patients. PTC124, now called *Ataluren*[®], is already being clinically tested against Duchenne as well as against cystic fibrosis. A number of other diseases, like some limb-girdle dystrophies, spinal muscular atrophy, and hemophilia are also caused to some extent by premature stop codons which might be influenced by drugs like Ataluren.

PTC has used high throughput screening also in other pharmacological approaches for finding therapies for all Duchenne patients independent of their dystrophin mutations. Here, with the GEMS technology – *gene expression modulation by small molecules* – the production of proteins is increased or decreased without the need to know the exact mechanism. Within *Project Catalyst*, several hits are now being optimized for the upregulation of the expression of utrophin, alpha7 integrin, mIGF1 and SERCA2a as well as for the downregulation of myostatin. All five procedures have shown positive effects in dys-

trophic laboratory animals, and clinical tests with patients are being prepared.

In the second presentation of this session, **Jenny Morgan** (University College, London) spoke about the **“Draw-backs and possible solutions for the use of myoblasts to screen dystrophin exon-skipping antisense oligonucleotides”**. Myoblasts are muscle precursor cells which are one step further developed than the satellite cells, the early “adult” stem cells of skeletal muscle. Myoblasts contribute to the regeneration and repair, of muscle tissue after injury, as they differentiate into myotubes and finally into functional muscle fibers.

Because myoblasts can be grown in cell culture, they can be used to test muscle therapies in the laboratory and also in living animals. For instance, the skipping of a targeted exon with an antisense oligonucleotide, AO, for a Duchenne therapy can be confirmed in pre-clinical experiments for each patient in a clinical trial in a cell culture of his own myoblasts before he is treated with his *personal* candidate exon-skipping AO drug. But a sample of the patient’s muscle tissue, obtained by a biopsy, is needed for the isolation of the myoblasts which have his mutation in their dystrophin gene.

The isolation of these Duchenne myoblasts is not easy, because during following purification based on specific marker proteins on their cell surface, they may change their properties in tissue culture and no longer differentiate into myotubes. They also multiply slower than normal myoblasts and often stop dividing too early. Because of these difficulties to maintain a myoblast culture with constant properties over some time, a second exon-skipping experiment can often not be performed to confirm the results of the first test.

For this reason, fibroblasts from a skin biopsy have also been used for these pre-clinical investigations. They are easy to isolate, but the gene for the marker protein MyoD has to be transferred into them by an AAV vector – they have to be *MyoD transfected* – before they can be used for exon-skipping experiments in which the sequence of the skipped mRNA can be determined but not well enough the structure of the dystrophin protein. Another possibility is the immortalization of myoblasts by the transfection with the enzymes telomerase and cyclin-dependent kinase 4. These long-living cell cultures allow the repetition of experiments in the laboratory and in living animals, but their preparation is labor-intensive, and the myoblasts from Duchenne muscles are often not sufficiently myogenic, i.e. they do not change easily into myotubes and muscle fibers.

Dr. Morgan finished her presentation by suggesting that immortalized Duchenne myoblasts and MyoD-transfected fibroblasts with defined mutations of their dystrophin genes should be available in biobanks to serve as controls for exon-skipping tests before clinical trials.

Rebecca Pruss (Trophos SA, Marseille, France) explained in her presentation with the title **Where is the target? Drug discovery and target identification applied to motor neuron diseases**, that her company is using high

throughput screening to find drugs for the motor neuron diseases *amyotrophic lateral sclerosis*, ALS, and *spinal muscular atrophy*, SMA. A possible cause of these two diseases is the loss of contact between the motor neuron and the muscle cell at the neuromuscular junctions, which starves motor neurons of growth factors provided by the muscle. This leads eventually to motor neuron death but the exact mechanism of the disease process, or what molecular target to use for a drug screening assay, is not known and doesn't have to be known.

For this reason, the Trophos scientists are using *living* motor neurons for the screening assay. Each well of the plate where the test reaction takes place contains 100 – 400 living cells. All processes following preparation of the cells are automated and data is collected using a newly developed analytical instrument called *Plate Runner HD*, which determines the number of living cells 2 to 6 days after the addition of the compounds to be screened by measuring fluorescent light at three different wavelengths. Motor neuron survival is detected by the ability to convert a substance added to the culture medium to a fluorescent intracellular product, which is the endpoint of the screening process. In other words: The number of motor cells kept alive at the end of the assay is an indication of the activity of the tested compound.

Among 40,000 tested compounds, including some approved drugs against other diseases and factors known to positively influence nerve cells, *six hits* were found. They were steroids and steroid-like compounds. For the optimization of the hits, 300 similar compounds were purchased or newly synthesized. One of them, TRO19622, also called *olesoxime* had the best properties as a drug candidate for motor neuron diseases. It is a lipophilic substance with a cholesterol-like structure that can be formulated into capsules. It is transported into cells and enters mitochondria, the energy producing organs of the cells, where it appears to reduce the consequences of oxidative stress there. Chemically it, can be synthesized easily in lots of 100 kilograms in clinical grade quality.

Olesoxime is now being tested in a clinical phase-III trial with ALS patients. It has completed a phase-Ib trial in SMA patients and a phase III trial will start in 2010. Olesoxime is also a drug candidate for *chemotherapy-induced peripheral neuropathy*, CIPN, a still untreatable side effect of cancer therapies. Another drug candidate is TRO40303, which is being developed to treat *cardiac ischemia-reperfusion injury*, IRI, and Trophos has other ongoing programs to find drugs to treat *multiple sclerosis*, *Parkinson's disease* and other neurological diseases.

Gideon Dreyfuss (Howard Hughes Medical Institute, University of Pennsylvania in Philadelphia) discussed in his presentation **SMN function and high throughput screening for SMA**, work to define the molecular function and find compounds that would upregulate the amount or activity of the *survival-of-motor-neuron* protein, SMN, in patients with *spinal muscular atrophy*, SMA, a severe and still incurable autosomally inherited disease.

The gene for the SMN protein on chromosome 5 exists in two copies, SMN1 and SMN2, both encoding the same SMN protein. However, the splicing of the messenger

RNA (mRNA) from the SMN2 gene is inefficient in producing full-length SMN mRNA due to a point mutation that causes skipping of exon 7. Full-length SMN protein is functional but SMN lacking the sequence encoded by exon 7 is rapidly degraded. This is inconsequential as long as the SMN1 gene is intact, but SMN1 deletions, which occur in most SMA patients, leave SMN2 as the only source of SMN and thereby to a deficiency in full-length SMN. Only about 20% of the SMN2-derived mRNA is full-length, functional protein and this is insufficient to maintain motor units, especially for the motor neurons with their very long axons and their neuromuscular endplates that connect them to the muscle cells. The consequence is SMA that manifest as clinical types of various degrees of severity depending on the remaining amount of normal SMN protein (which correlates with SMN2 gene copy number).

Dr. Dreyfuss described studies that revealed a critical function of SMN protein in the biogenesis of the splicing machinery in all cells. Studies have now dissected the biochemical pathway whereby SMN together with its associated proteins (called gemins) build the subunits that cells use to splice pre-mRNA to produce mRNAs. Insights from this basic research and the tools generated in the course of this work suggest new approaches to increase SMN protein or compensate for the loss of SMN's activity in SMA patient cells.

One way to a therapy of SMA would be the upregulation of the amount of SMN protein from the SMN2 gene in the patients. Dr. Dreyfuss described the SMN2 gene as "a therapeutic opportunity" and outlined several approaches he and his colleagues developed towards finding a therapy for SMA. Notably, using one such technology to search for compounds that may increase full-length SMN protein in SMA patient cells – by any mechanism – the large pharmaceutical company Merck & Co tested close to 1.2 million compounds in an automatic high throughput screening program performed in a joint project between industry and academia to help Dr. Dreyfuss and his team to develop an SMA therapy. For this screening, a cell-based immunoassay, CIA, was developed to detect the SMN protein directly in fibroblast cells obtained from a skin biopsy of an SMA child. Hits from the primary screen were then selected by a matrix of additional screens and assays. Examples of several select hits illustrated the promise of this effort and pointed to the large amount of work that has been done and that will need to be done to develop these leads. The collaboration with Merck & Co is continuing and efforts are also underway to look for compounds that would influence other processes responsible for the clinical symptoms of SMA patients.

In an example of another large primary screening program on more than 65,000 compounds Dr. Dreyfuss's laboratory identified compounds that modulate the biochemical activity of the SMN-Gemin complex. To date this identified oxidative agents as inhibitors that may paralyze the SMN complex. These compounds provide invaluable research reagents for understanding the regulation of the SMN complex, suggest ways to protect it from environmental damage, and could lead to design of upregulators that can become drug candidates for clinical trials with patients.

Animal model assessment

One of the most significant challenges to the development of new and promising therapies for human disease is the “translation from the laboratory into the clinic”. The challenge is to take treatments that appear to be effective in preclinical experiments with laboratory animals and to test the efficacy of these treatments in human clinical trials. In this section **Michael Benatar** (Emory University in Atlanta GA, USA) in his presentation **Lost in translation: lessons for ALS from the SOD1 mouse** asked first whether the most commonly used laboratory animal for testing possible drugs for treating human *amyotrophic lateral sclerosis*, ALS, the SOD1 mouse, really is a good model of the disease for the purpose of predicting how patients with ALS would respond.

ALS is the most common motor neuron disease which affects mainly people between the ages of 40 and 70 years, and about half of them die 3 to 5 years after diagnosis from a general paralysis of all muscles. In Europe and in the United States, about 75,000 ALS patients are living at any time. There is no effective therapy, the only approved drug, Riluzole[®], prolongs life only modestly. It was found before the ALS mouse model was available and has only a very modest effect, if any, on survival of the SOD1 mouse.

About 90% of the patients have the sporadic form of ALS and 10% have the familial form of which 20% have a mutation in the SOD1 gene for the enzyme *superoxide dismutase 1*, which destroys the toxic oxidative agent hydrogen peroxide. The laboratory animal most commonly used as a model of ALS, is based on the introduction of mutant human SOD1 into the mouse. Although the SOD1 mouse recapitulates several aspects of human ALS, there are many important differences. For example, this mouse model is genetically relevant to only 2% of all ALS patients and it results from a massive overexpression of the SOD1 protein in a way that is not comparable to the human form of the disease.

Apart from the question of the suitability of the SOD1 mouse as a model for the preclinical evaluation of potential therapies for human ALS, questions about the methodological quality of many of these preclinical therapeutic studies make it very difficult to interpret the results. Moreover, many of the mouse therapeutic studies with negative results have not been published. So it is still impossible to say whether results from experiments with the SOD1

mouse are predictive for what might happen in a human clinical study. Thus, an animal model of ALS, that is reliably predictive of beneficial effects in humans with ALS, is still not available.

Discussion: The ideal animal model is one that duplicates the human disease completely or almost so. The SOD1 mouse is not an ideal model for ALS. The mdx mouse that has no dystrophin but not the severe symptoms of Duchenne boys is a much better model and, therefore, is being used extensively in preclinical experiments. The double-KO mouse that has neither dystrophin nor utrophin and four other such double mutant mice have Duchenne-like symptoms, but because their mutations are different of those in Duchenne boys, they are less useful than mdx mice to predict therapeutic effects in human patients.

The dystrophic GRMD dog is very valuable for Duchenne dystrophy studies because of its size, so drugs can be tested with dosages similar to those later needed for Duchenne boys. Similar positive results with both animals, the mdx mouse and the GRMD dog, give the researchers confidence that the drug tested will bring the same results in boys. For the same reasons, pigs with SMA are now being developed.

Clinical trials with human patients should only be started when relevant animal experiments have shown significant efficacy, not just borderline results. All the results of preclinical studies with animals, also those with negative results, should be published.

TREAT-NMD is developing standard operating procedures, SOPs, for testing drugs and treatments in laboratory animals: www.treat-nmd.eu/research/preclinical/SOPs/. Following such standards would avoid costly mistakes and allow easy comparison of results from different laboratories. The experiments should also be done according to GLP guidelines, which describe accepted general laboratory practice.

The laboratory animals must be treated with great care as humanely as possible, and surgery must be performed with anesthesia. The scientists should actively explain to the public how they care for their animals, how they do experiments with them, and why they are necessary to find therapies for still incurable diseases with often completely new techniques when alternatives are not available

Therapeutic misconception and ethical consideration.

Simon Woods (an ethicist from Newcastle University, and TREAT-NMD collaborator, UK), chaired a debate and panel discussion in what was – in my opinion – the most important session of the meeting. Dr. Woods began by emphasising that the participation of patients with rare diseases in clinical trials is absolutely necessary and must be based upon voluntary and adequately informed consent. However, there is evidence that parents, who have to give this consent on behalf of their children, may be unable to distinguish between research and treatment. The so-called *therapeutic misconception* may exist when the participants of a clinical trial believe that its central purpose is to pro-

vide a therapeutic benefit.

But clinical trials are just one step in the *scientific process* on the way to an effective therapy. Dr. Woods defined science as being “concerned with making cautious claims of knowledge based on the best possible evidence and balanced by a healthy willingness to re-evaluate this knowledge in the light of new evidence”. Therefore, a clinical trial is *only a scientific experiment* that does not guarantee positive results, and in spite of carefully performed preclinical experiments done before a trial is started, it may still involve unknown risks. This is particularly true of the early phase-I and phase-II trials which are designed to

check whether the new procedure is safe. Any clinician inviting patients to participate in a trial must consider the requirement of the Hippocratic Oath to do no harm. Thus, at this early stage, a therapeutic benefit cannot be expected and is sometimes impossible, for example if only a single muscle is treated.

The scientists and clinicians performing the clinical trial must do their utmost to inform the parents of a child with a muscular disease about all details of the trial. But if after these explanations the parents are still unable to distinguish between research and treatment, and say that their agreement to participate is based on an unrealistic hope for a therapeutic benefit, then this misconception may undermine their ability to give their *voluntary and informed consent*. This problem was put to the audience as the starting point for debate and discussion. The following motion was proposed:

Parents who express hope in the possibility of therapeutic benefit from clinical trial participation should not be allowed to consent for their children to enter trials.

I am reporting here in abbreviated account of the ensuing complex and at times emotional 70-minute-long discussion between the audience and the members of the invited expert panel. The debate was very wide ranging and with three exceptions at the end, I could not identify by their voice in the recordings those who made the statements.

A number of important points were made addressing the responsibilities of the researchers: The scientists performing a trial should explain the details of the study in a relaxed conversation, in ordinary language and with plenty of time, “just sitting there with a cup of coffee”. They should speak slowly, sentence by sentence, using ordinary words without medical or other scientific expressions. The parents should be asked to summarize how they understood what was said and this would provide an opportunity for the researcher to correct any misunderstanding.

The risk that something serious and unexpected might happen must be discussed quite openly. For example animal studies have some limitations in terms of predicting when moving to human studies. After all, the mdx mice are all related. They belong to one family, so the results of experiments with them can be less variable than those from clinical trials with humans who are so different from each other. Results of human trials are not 100% predictable; *a boy is not a big mouse and no dog either!*

It is not unusual for a special personal relationship to develop between the families and their specialist doctor. Often this specialist also belongs to the team performing the trial. The families will then trust their “carers”, knowing that they always will act in the best interest of the patient. Under these conditions, the families will often accept the doctor’s and sign the consent form on trust without long explanations but with a risk that they do not fully understand the study and the risks. Families and doctors should therefore be careful to ensure that the details of a study are discussed in detail.

Families with older sick children, who have experience of clinical trials, may be able to help those whose children are newly diagnosed to understand the issues. The parents associations can also be of great help, but they should be cautious not to simply encourage participation in research

but enable parents to gain accurate and detailed information so that they can make up their own minds.

Also, a standardized consent form, or at least a template with good examples of clear language would be of help. Many researchers would also welcome examples of texts which seek to explain the important aspects of clinical trials in parent and patient friendly language.

A very important aspect of the discussion addressed the view that “hope” is a natural condition of the human spirit and that it does not disqualify someone from rational thinking. Patients do not have time to wait for many years, and many feel that their time is running out, leading some to regard participation in a trial as last hope. It was also noted that even those who recognize there is little chance of personal benefit – like a 20-year old young man with Duchenne did – may still want to participate in research out of a wish to help the ones coming “behind them” by taking part in a trial. Hope for a personal benefit isn’t always the only reason for trying to be in a trial. The altruistic wish to help other patients or the NMD community in general may also play a role. Nobody should be judged because of his or her motives to participate.

While writing this report, it occurred to me that there is another reason for hope: If at the end of a trial the preliminary results are considered positive, there is sometimes an *open extension* during which all participants, also those who got a placebo, can receive the drug tested before its official approval. This, I think, might be a strong reason of hope for a therapeutic benefit.

Nick Catlin, president of ActionDuchenne, who has a 9-year old boy with Duchenne, said: “We are hurt by the disease and therefore are more “vulnerable” than those with healthy children, but that does not mean that we are mentally unstable. We can still make rational and clear decisions for our children. Starting at the day we heard the terrible diagnosis, we are struggling with the death sentence of a child who is still running around like a normal one. We have ups and downs, on some days we can cope with this situation, but there are other days when we just want to cry.

But we don’t lose our faculty to think. We know the difference between risk and benefit. But we are not always given the full facts and often not taken seriously. John Porter was right when he said that in the new era of personalized medicine there must be partnership between us and you, the scientists and clinicians, with us the central part of it. If you don’t include us, you are going to fail. But we do not want to lose this chance of taking part in going ahead towards a therapy.”

The president of the American Parent Project Muscular Dystrophy, PPMD, *Patricia Furlong* told us that her two boys, Christopher and Patrick, died of Duchenne in 1995 and 1996 and then continued: “They had participated a few years earlier in the unsuccessful clinical studies with the myoblast transfer technique. It had been a frightening experience, and I wondered, what is the therapeutic dose of hope, that we are allowed to have?”

We want the best for our children from the day they are born, and Duchenne does not change that. We want to be equal partners with you, the scientists and clinicians, and we need to be informed in an active ongoing dialogue about all the details of a trial with words we are able to

understand. We need the dialogue to express our concern and to hear your response. We will tell you about our burden, because you do not live in our home 24 hours a day. It is very different from what you imagine! We all have our hope, but you have hope, too, I know, otherwise there would be no research and no clinical trials. For our children we need the light at the end of the very long tunnel”.

Dr. **Karl Bettelheim**, a director of ActionDuchenne and grandfather of Frederick, a 7-year old Duchenne boy, used quite drastic words to make his opposition to the motion clear: “This proposal is, in my opinion, a threefold insult. First of all, it is an insult to the scientists performing the clinical trial because it implies that they will not do it sufficiently well. Secondly, it is an insult to the parents, because it implies that the parents would attempt to falsify the results of the trial by subjecting their child to some undue pressure. And finally, it is an insult to the children, because it would imply they would only participate in a trial because they expect a possible benefit from it.

One needs to look at the logical conclusion of this proposal: Only parents who do not express hope in the possibility of therapeutic benefit from a clinical trial, should be allowed to consent for their children to enter trials. But I think, parents who do not express hope, would be very difficult to find. So, I have made up a statement of clinical-trial correctness: This is a doctrine fostered by delusional ethicists, who hold forth the proposition that it is entirely possible to make themselves look like the only judges of morality and ethics, without regard of whether the parents who want the best for their children are morally and ethically harmed.

To make clear what I mean, let me describe a scene from a BBC film that plays in May 1941, for which I acted as scientific advisor. In an Oxford hospital, Johnny Cox, a 4-½-year old boy, lies in a coma. On one side of his bed sits his father, Mr. Cox, on the other side sits the clinical scientist who says to Mr. Cox: “There are no guarantees,

Mr. Cox, the last patients we tried this drug on, didn’t make it.” Mr. Cox answers: “I know you are not promising a miracle, I understand that”. The next day, there was a significant improvement. The clinical scientist said: “We will give him the full 4-week treatment even if he appears to be fully recovered, we don’t stop.” Three days later, despite of appearing well, he had convulsions and died within ten minutes. A post mortem revealed, that the boy did not die of the bacterial infection that he had, he died of a ruptured carotid artery, caused by the infection. The drug he was given was penicillin and this was the first clinical trial with this first antibiotic.

I would say that anybody in this room, who has benefited from being treated with penicillin or any antibiotic discovered subsequently as a result of the great discovery of penicillin, is duty-bound to vote against this atrocious motion.”

And so it was done! We were asked to hold up our blue cards if we supported the motion and our yellow cards if we were against it. After a few moments, the result was clear: About 98% of all the people in the audience held up their yellow cards. It was not necessary to ask the supporters to show their blue cards.

Dr. Woods added the following conclusion to my summary: “The debate was complex, detailed and often emotional, however a number of conclusions were drawn. It was recognized that researchers need to work in close partnership with parents and patients. It was observed that hope is an important and necessary part of coping with these diseases, but hope should not blind parents and patients to the real risks of participation in research. More work is needed to enable collaboration and joint working to high standards of practice in research, to develop good quality information, and to guarantee that the best possible methods are used across the globe to enable safe and ethical participation in research.”

Developing novel, disease targeted therapies and systemic delivery.

The keynote lecture for this session: **New treatments for hereditary neuromuscular diseases** was delivered by **Kenneth Fischbeck** (National Institutes of Neurological Disease and Stroke, NINDS, Bethesda near Washington DC, USA). Dr. Fischbeck discussed the therapeutic challenges of muscular dystrophy and opportunities for therapeutic intervention.

Pharmacological approaches include agents such as Ataluren® from PTC Therapeutics that correct the translation of the mutant mRNA and agents that enhance muscle regeneration such as myostatin inhibitors. Gene replacement therapy has been pursued for Duchenne muscular dystrophy with adenovirus, adeno-associated virus (AAV), and direct plasmid injection in animal models with some benefit, although a marked inflammatory response occurred in dogs with AAV delivery. A human trial of AAV delivery of truncated dystrophin is currently being done by Dr. **Jerry Mendell** and his team at the Nationwide Children’s Hospital in Columbus OH, USA.

The same group also recently reported the first successful clinical trial of gene delivery for *limb girdle muscular*

dystrophy type 2D which is caused by the loss of *alpha-sarcoglycan*, SGCA, one of the proteins of the dystrophin-glycoprotein complex that anchors one end of the dystrophin to the muscle cell membrane. Dr. Mendell’s team transferred the short SGCA gene for this protein – only 9,927 base pairs long – with an AAV1 vector by local injection into the small EDB foot muscle of three patients. After 6 weeks, the protein level had increased 4-5-fold over the control level, and the dystrophin-glycoprotein complex was restored. Some immune response against the virus vector was seen, which means that immune reactions must be taken into account in future clinical trials. The details of this trial were published in September 2009 in the journal *Annals of Neurology*, volume 66, pages 267-270. Presentations on gene delivery for muscular dystrophy were given by Drs. **Jon Wolff** and **Luis Garcia** in this session and are summarized below.

Dr. Fischbeck also discussed the development of exon-skipping oligonucleotide therapy for Duchenne dystrophy. Preclinical studies in the mdx mouse model were encouraging, and clinical trials of local injection were published

in 2007 and 2009 by groups in the Netherlands (working with Prosensa) and the United Kingdom (working with AVI). Subsequent studies by both groups were presented by Drs. *Judith van Deutekom* and *Francesco Muntoni* in this session.

Dr. Fischbeck also discussed research to develop a therapy for spinal muscular atrophy, SMA. With histone deacetylase, HDAC, inhibitors, the quantity of SMN protein and the survival time of SMA mice were significantly increased. High-throughput screening has identified active compounds that promote retention of exon 7 in the mRNA produced by the SMN2 gene, and oligonucleotides developed by Dr. *Adrian Krainer* are even more effective at doing so. These compounds have been found to increase SMN protein levels which then improved the clinical symptoms of the SMA mouse. Recent preclinical results with AAV-mediated delivery of the SMN gene by Dr. *Brian Kaspar* and others have also been very encouraging and were reported at the end of this session.

As explained in other presentations, the SMN protein plays an important role in the spliceosomes of all cells. But it is probably also necessary for transporting the mRNAs through the long axons of motor neurons to growth cones and neuromuscular junctions at the connection to the muscle fibers, where they are needed for the synthesis of proteins. Thus, an insufficient amount of the SMN protein interferes with the transfer of the nerve signals to the muscles and thus causes the muscle weakness and atrophy of SMA.

At the end of his lecture, Dr. Fischbeck said that finding therapies for neuromuscular diseases has proved to be more difficult than thought over 20 years ago when the dystrophin gene was found. But with the new research tools and the growing involvement of pharmaceutical and biotechnology companies, the barriers to safe and effective treatment will be overcome.

Following this introductory lecture, the four clinical trials with the exon-skipping technique were discussed, the two local ones whose results have been published and the two systemic ones who are finished or almost finished, but whose results are not publicly known yet. The skipping of exon 51 was chosen for these first trials, because 13% of all Duchenne patients – the largest group who needs skipping of one single exon – would benefit from skipping of this particular exon. I am summarizing here the rather short presentations by Drs. *Judith van Deutekom* and *Francesco Muntoni*. More details of all four trials are described in my recent report on exon skipping, updated in October 2009, which can be seen on the internet at www.duchenne-information.eu/Exon-Skipping-Report-English-firstupdate-October-2009.pdf.

In her presentation **New results from Prosensa's exon skipping trial** *Judith van Deutekom* (Prosensa Therapeutics, Leiden, the Netherlands) said that it is actually quite amazing that when compared to typical drug development programs taking at least 10 years, it took scientists together with Prosensa and AVI only 8 years: from the first proof in 1998 of a successful skipping of an exon in cultured muscle cells from a Duchenne patient and in the mdx mouse model until the beginning of the *first in-man* and *proof-of-*

principle clinical trials of this new genetic technique with Duchenne boys in 2006.

Prosensa's first trial was a *local* study performed between January 2006 and March 2007. The modified antisense oligonucleotide (AO) *2'-O-methyl-phosphorothioate* AO PRO051 against exon 51 of the human dystrophin gene was injected into one shin muscle of four Duchenne boys. After four weeks, up to 95% of the muscle fibers were dystrophin positive. This treatment was proven to be safe without side effects, but no therapeutic benefit for the children could be expected. The full results were published in December 2007 in the *New England Journal of Medicine* on pages 2677-86 of volume 357.

Prosensa's first *systemic* exon-skipping trial was performed in Leuven and Gothenburg between April 2008 and May 2009. Twelve patients received PRO051 in 5 weekly subcutaneous injections. This was a dose-escalation study with doses between 0.5 and 6 mg/kg. New dystrophin without the amino acids coded for by exon 51 was detected in a dose-related manner without side effects. The main purpose of this trial was to determine whether this first whole-body treatment was safe. But muscle function tests were also performed because there was a chance that the boys would already obtain a therapeutic benefit. Dr. van Deutekom was not allowed to mention the full results of the study. They will be known after publication within the next months. All 12 patients participate in an *extension* of this study with weekly injections of the highest dose for at least 6 months. No drug related side effects have been detected so far.

Francesco Muntoni (University College, London, UK) discussed in his presentation **New results from AVI's exon skipping trial** the two clinical trials with another type of modified AOs, the *morpholino* AO against exon 51. In their *local* study between the autumn of 2007 and the end of 2008, the British scientists treated the small foot muscle EDB in one foot of seven Duchenne boys with their morpholino AO AVI-4658 against exon 51, developed by the MDEX Consortium in collaboration with *AVI Biopharma* in Bothell near Seattle WA, USA. Four weeks after the injection of 0.09 or 0.9 mg of the drug, up to 42% of the fibers around the injection site contained the new but shortened dystrophin in the 5 children who received the higher dose; there was no significant protein production in the 2 boys who received only the low dose, which was expected. As in the local Dutch study, no therapeutic benefit could be expected, and no side effects appeared. All details of this study were published online in August 2009 by the journal *The Lancet Neurology* and then printed in volume 8 on pages 918-928.

The British *systemic* trial started in February 2009 in London and Newcastle, it will be finished in March 2010. A total of 18 boys are scheduled to receive escalating doses of the drug between 0.5 and 20 mg/kg/week for 12 weeks injected intravenously. Two weeks before and two weeks after the injections, biopsies were performed for the analyses of dystrophin mRNA and protein. Again, as in the Dutch systemic trial, safety was the main outcome measure, but muscle function was also monitored because the patients may get a therapeutic benefit. No drug-related adverse effects have been detected. The full results will be

known after their publication in the second half of 2010. However the analysis of the first 12 treated children identified the presence of skipping and dystrophin production in at least some of the treated patients.

Discussion: I could not identify by their voice all panel members who answered.

Exon skipping is the most advanced research approach towards a Duchenne therapy.

Judith van Deutekom: Prosensa has tried both systemic (intravenous and subcutaneous) delivery methods on mice and monkeys. They are similarly effective, but the subcutaneous one minimizes risk for potential toxicity and would be more practical later because it could be applied at home without a visit to a doctor's office or hospital.

Annemieke Aartsma-Rus: Exon skipping will not be able to replace lost muscle fibers but only maintain and stabilize those that are still present. Thus wheelchair patients will not walk again, but may probably continue to use their hands and arms longer.

Judith van Deutekom: No serious side effects of exon skipping with both AO types have appeared. But only time will tell whether there will be long-term risks. Prosensa always checks the AO sequences for off-target effects to avoid that they would bind somewhere else in the human genome outside the dystrophin gene.

Both types of AOs typically do not cross the blood-brain barrier. Thus skipping would not affect the dystrophin in the brain which is shorter than normal and might be responsible for mental problems of some patients.

Exon skipping of cardiac muscles is not yet sufficiently efficient. We might create Becker-type patients with severe heart problems. *Judith van Deutekom:* 2OMePS AOs are taken up by the heart, at lower levels but with longer half-lives!!

Judith van Deutekom: An AO drug for skipping exon 51 will be the first one to be approved by FDA and EMEA. It will take another few years. The next ones will come in short succession. *Francesco Muntoni:* We are much closer than ever at approved exon skipping drugs. I was always wrong when I gave a time estimate. My (GS) personal addition to this debate: In my interview with *Gertjan van Ommen* in 2004 in Monaco, he said it will take about 10 years until exon skipping will be ready for our boys.

Jon Wolff (University of Wisconsin and Roche Madison Inc., Madison WI, USA) discussed his experiments with "naked DNA" in his talk **Limb perfusion gene delivery**.

Plasmids are small circular DNA structures without protein inside bacteria to which they mostly confer resistance against antibiotics. To test whether these naked DNA structures could be used as vectors for gene transfer into muscles, the scientists inserted the combined 79 DNA exons, the entire cDNA, of the dystrophin gene together with controlling sequences into plasmids and injected this vector system under pressure into the blood stream of the hind legs of mdx mice. The pressure was produced by short-term blocking the blood circulation with a tourniquet, a blood pressure cuff. Repeated treatments led to the stable production of up 20% of the dystrophin level of normal mice in about 15% of all muscle fibers in the treated limb which lasted for the remaining life of the mdx mice. This

hydrodynamic procedure was well tolerated by the mice, whose muscle function of the treated limb showed a significant improvement.

In preparation of a possible human application, Dr. Wolff and his colleagues then tested this regional gene therapy on non-dystrophic monkeys. After a single injection of plasmids carrying the gene of the enzyme beta-galactosidase, 30 to 40% of the muscle fibers in the treated limbs contained this marker protein. The treatment under pressure produced a swelling of the muscles which, however, disappeared after about 24 hours. More than 100 monkey limbs have been treated that way without any harmful side effects.

The first human experiments with three healthy adults have now been started who are receiving injections of salt solution to test whether this method could become a routine method for treating Duchenne children.

A next-generation exon-skipping technique would be one that would require just one single injection of a drug without the need for repeated follow-up treatments for the remainder of the patient's life. Such a treatment was developed by Dr. *Luis García* at the Institute of Myology of the Pierre and Marie Curie University in Paris and Dr. *Aurélie Goyenvalle*, now at Oxford University, and their colleagues. With this technique, the gene of a modified splicing factor U7 with the antisense sequences against the targeted exon attached are transferred into the muscle cells with an adeno-associated virus, AAV, as vector so that *the cells themselves can make the exon-skipping drug* continuously. I have described also the details of this AAV-U7 technique in my exon-skipping report, whose internet address is shown at the beginning of this section.

At the meeting, **Luis García** in his address entitled **New results from systemic AAV total-body delivery**, presented the first results of his experiments to *translate* the studies on mdx mice to the much larger dystrophic GRMD dog. The experiments with mice had shown that two months after the injection of the AAV-U7 system into their tail vein, most of the fibers of almost all muscles, also those of the heart, contained the intended shortened dystrophin without the amino acids coded for by exon 23.

The U7 system, containing antisense sequences against the exons 6 and 8 of the dog dystrophin mRNA for restoring the reading frame after the dog's deletion of exon 7, was then used for the regional treatment of one leg of a GRMD dog by the hydrodynamic regional delivery method described by Dr. *Jon Wolff* in the previous presentation.

After positive results were obtained after this regional treatment, a *full systemic delivery* through extracorporeal circulation powered by a pump was performed on the dystrophic dog *Droopy*. Two hundred milliliters of a solution containing 270 trillion (2.7×10^{14}) virus particles were delivered in 4 portions within about 5 minutes. A cardiopulmonary bypass was used to avoid as much as possible the lungs.

Two months later, wide-spread distribution of the dystrophin mRNA without the exons 6–9 and the correspondingly shortened dystrophin protein was detected in all muscles analyzed in biopsy material. The skipping of exon 9 was not intended, but the removal of this extra exon does

not change the reading frame. The dog supported very well the procedure, but the amount of the newly synthesized dystrophin was too low to have an effect on muscle function despite the large amount of vector infused. Possibly the vectors were blocked by something in the blood stream and subsequently could not get out of the blood vessels and into the muscle tissue. Studies are going on to bypass this crucial bottleneck and to obtain an improvement of the muscle function before clinical trials with Duchenne boys can be contemplated.

In the last presentation of this session, **Brian Kaspar** (Nationwide Children's Hospital, Columbus OH, USA) spoke about **Gene therapy for spinal muscular atrophy (SMA)** for which the largest problem was the systemic delivery of the SMN protein to the motor neurons residing in the spinal cord. Despite tremendous efforts by researchers over the last 10 years, systemic delivery to the central nervous system – CNS, brain and spinal cord – has been a challenge. Dr. Kaspar reported, that the researchers have now found that type-9 adeno-associated viruses, AAV9, could be used as a vector which could carry the gene for a green fluorescent marker protein, GFP, or the gene for the full-length SMN protein across the blood-brain barrier in *newborn* mice, the barrier which protects the CNS from harmful substances. The viruses with their charge were able to enter large numbers of neurons throughout the entire brain

and many motor neurons in the spinal cord, where the GFP marker or the full-length SMN protein was produced. In treated *adult* animals, only astrocytes – the cells that surround the nerve cells and blood vessels in the CNS – and only small numbers of neurons were making GFP. In addition, the vectors entered also skeletal and cardiac muscles in both newborn and adult animals.

Preliminary experiments were performed with the animal model for severe Werdnig-Hoffmann SMA type I, the SMN2-delta7 mouse. As in human patients, this mouse has no functional SMN1 gene, and the mRNA of its SMN2 gene has a deletion of exon 7. With systemic injections of the AAV9-SMN vector system into young, 5 – 13 days old mice, the researchers showed that while untreated SMA mice live only 15 to 20 days, the animals treated during that time window had no SMA symptoms anymore and lived for longer than 100 days. However, the treated animals grew later to only half the size of normal mice.

The ability of the vector system to efficiently enter motor neurons after intravenous injection was also repeated in other laboratories. One research group in France led by Dr. *Martine Barkats* used AAV9 in SMA cats and obtained similar results.

The next steps are experiments with non-human primates, for instance monkeys, to test the applicability of this approach and to demonstrate safety, before clinical trials with SMA children can be contemplated.

Registry development for clinical trials.

As more and more clinical studies are being performed and prepared for the development of therapies of neuromuscular diseases, *patient registries* are becoming important for finding participants for these studies with well-defined genetic diagnoses and up-to-date clinical data in reach of trial centers.

The three speakers in this session discussed as successful examples mainly the global international registries for Duchenne muscular dystrophy, DMD, and spinal muscular atrophy, SMA. The two registries can be reached at the internet address www.treat-nmd.eu/patients/patient-registries/global-registries/. Through the TREAT-NMD address, 19 national DMD registries, 17 SMA registries, and 8 registries for myotonic dystrophy type 1 can be reached. All these registries are connected to each other in the TREAT-NMD registry network. Therefore, DMD and SMA patients need only to be registered in one, preferably their own national registry, because their most relevant data, *the minimal data set*, is sent to the global DMD or SMA registry. The global DMD and SMA registries are located at the University of Montpellier in France. Patients in countries without a national registry can have their data entered in one of the registries that use online self-report forms.

The following description of the advantages and benefits of patient registries is based on all three presentations in this session:

(1) The registries act as a liaison between patients and families interested in participating in research and researchers in universities and industry interested in studying their particular disease and developing therapeutic proce-

dures.

(2) They allow for more rapid movement of studies from conception to clinical trial. Thus, they promote the effective use of funds.

(3) Data about the medical management of patients will help to develop and maintain good standards of care according to internationally agreed recommendations.

(4) Registered patients and their families feel that they are not left behind but belong to their disease community, because they are getting up-to-date research and care information, especially about the begin of clinical trials which might need their participation.

(5) As the clinical course of a patient's disease from its onset in the past to the time during the treatment after the approval and marketing of "their" drug can be documented continuously, this data can be used to study the natural history of a disease for control purposes and for the detection of any side effects and additional effectiveness data that develop during the long-term use of the drug.

(6) The availability of past and future patient data can convince the regulatory agencies to approve a drug for a rare disease even when safety and randomized studies cannot be performed as required for the approval of "normal" drugs.

In her presentation **Huntington disease and SMA** **Jaqueline Jackson** (Indiana University, Bloomington IN, USA) told us that some of the most important registries have begun their work many years ago: The registry for Huntington disease in 1979, for SMA in 1986, for Alzheimer disease in 1990, for Parkinson disease in 1998, for Charcot-

Marie-Tooth disease in 2001, and for familial intracranial aneurysm in 2002. Registries for many other neuromuscular diseases are listed at the registry pages of TREAT-NMD. The Indiana-based SMA registry contains now data of 2,300 SMA patients from 2,160 families. The registry for Huntington disease lists data of 14,000 patients from more than 3,000 families with 138,000 people.

For researchers to get access to the patients and their data, they have to submit a proposal of their research project which will be reviewed and then must be approved by a scientific advisory committee.

These registries have allowed thousands of families to participate in research and clinical trials and have led to thousands of publications about these diseases.

Per Nilsson (Actelion Pharmaceuticals, Allschwil near Basel, Switzerland) described in his presentation **Regulatory and industry perspective** the advantages the patient registries bring to industrial research. He mentioned among examples, *Myozyme*[®] against Pompe disease by Genzyme and *Zavesca*[®] against Niemann-Pick disease type C by Actelion. The development of these two drugs took a long time and was very expensive. If a long-term registry for these rare diseases had existed before the work for an effective treatment had started, the two drugs might have been available faster and at lower costs.

Another example is the drug *Tracleer*[®], now marketed by Actelion against pulmonary arterial hypertension, PAH. Tracleer was approved in 2002 in the EU with data on 170 patients in two randomized trials under the condition that a post-marketing risk-management program was established which required that physicians report any adverse effects on treated patients to Health Authorities. This was requested because the clinical studies had shown that about 11% of patients develop increased but reversible liver enzyme levels. This effort resulted in a PAH registry which after four years contained data of almost 5,000 treated patients,

covering 80% of prescriptions in the EU. This kind of registry has become a model for similar programs of targeted surveillance for other rare diseases.

A registry and patient study for the use of a thrombolytic therapy to dissolve blood clots in ischemic stroke was started in 2000 in Sweden and eventually included 11,000 patients from 30 countries. This registry was able to provide important data to show that the benefit of a thrombolytic therapy in ischemic stroke indicated by placebo-controlled trials could be translated into clinical practice. This again illustrates the great importance of patient registries for the development of drugs for severe diseases.

Hanns Lochmüller (TREAT-NMD, Newcastle, UK) finished this session with his presentation **TREAT-NMD patient registries**. In addition to information included in the introduction of this summary, he mentioned that it took three years to recruit the 150 Duchenne patients for the large German study on the treatment with prednisone combined with cyclosporine led by Professor *Rudolf Korinthenberg* at the Children's Hospital of Freiburg University, which showed that the addition of cyclosporine did not increase the effect of prednisone alone. At that time, no Duchenne registry was available which would have made the recruitment of the patients much easier and faster.

A reliable genotype-phenotype correlation would be of great practical importance because it would, for instance, allow to predict which symptoms of a Becker dystrophy a particular exon skipping drug would create. The genetic and clinical data from a DMD/BMD registry would facilitate the establishment of such a correlation.

TREAT-NMD is prepared to help parents associations to set up registries for their neuromuscular diseases. The European Neuromuscular Center, ENMC, in the Netherlands has also helped to set up and to run patient registries in cooperation with TREAT-NMD.

Clinical outcome measures.

Outcome measures are tools for assessing changes in a patient over time. They measure change in meaningful areas of a person's life in a way that makes decisions about treatments possible. I found this definition on the internet, and the program of the meeting contains the following three sentences of introduction to this session: The correct choice of outcome measures for a clinical trial can be critical to its success. Making these choices can be a time-consuming and lengthy process, and if the choices have been made, there are many practical issues that need to be addressed in order to successfully implement an outcome measure for a trial.

The following three presentations were held in this session about how to choose outcome measures, how to implement and optimize them, and how to assure their quality: **Michael Rose** (King's College, London, UK) **How should we choose outcome measures for clinical trials and studies?** **Julaine Florence** (Washington University, St. Louis MO, USA) **Implementing and optimizing the clinical outcome measures.** **Jeremy Hobart** (Peninsula Medical School, Plymouth, UK) **Quality assessment of outcome measures.**

Very many details were given by the speakers which, in my opinion, may not really interest the majority of the patients with neuromuscular diseases and their families for whom I have written this report. And as my expertise is research for therapies of Duchenne muscular dystrophy, I have difficulties reporting about this special technical field and summarizing the most important facts. For this reason, I ask those, who need to know these facts, to consult the detailed documentation on outcome measures published by TREAT-NMD on the internet: www.researchrom.com.

However, there is one outcome measure that is worth mentioning here: the *6-minute walk test* which is now widely used in trials for Duchenne and other muscle diseases with patients who can still walk. The first scientific study of this test with Duchenne and healthy boys was published online by the journal *Muscle & Nerve* on 25 November 2009 by *Craig M. McDonald* and co-workers of the University of California in Davis. The study results indicate that young Duchenne boys can consistently and reliably perform the test. The study was sponsored by PTC and supported by a grant from the American Parent Project Muscular Dystrophy (PPMD).

Effects of long-term treatment and combination therapeutics.

Robert Griggs (University of Rochester NY, USA) started this session with his presentation on “**Long-term corticosteroids in DMD: Implications for new treatments**”.

The treatment with one of the corticosteroids prednisone and deflazacort is presently the only drug treatment proven to be able to preserve or maintain the muscles of Duchenne boys for a limited time. This type of treatment is now considered the “gold standard” to which other pharmacological treatments in development are compared. But there is still disagreement about the answer to many questions: Which is the best steroid to use at which dose and with which administration schedule, what is the best age to start, should the dose be changed when the patient gets older, how long can the treatment be continued, what are the side effects and how can they be avoided or minimized? A survey has shown that 31 different treatment regimens are in use in 60 clinical centers worldwide. But many Duchenne patients still do not receive any steroid treatment.

A large international clinical trial, to be financed by NIH, is now being prepared which should answer many of the questions mentioned and whose results should lead to a proposed standard steroid treatment with defined characteristics. This trial will be performed in 40 clinical centers in 11 countries. The 300 participating 4-7-year old Duchenne boys in 3 randomized groups of 100 patients each will be treated for three years with 0.75 mg/kg/day prednisone or 0.9 mg/kg/day deflazacort with daily application or with an adjusted dose in the “Dubowitz regime”, 10 days on/10 days off. Many standardized outcome measures will be used so that the results from the different centers will be comparable. In cooperation with TREAT-NMD, a standardized management of side effects and a long-term follow up are planned for at least 10 years to detect short- and long-term positive and negative effects.

In his representation **Long-term side effects of drugs in development for NMDs**, **Rudolf Korinthenberg** (University Children’s Hospital Freiburg, Germany) said that in clinical trials with newly developed drugs, only the short-term side effects become known. But drugs for neuromuscular diseases will have to be taken for a long time, so during the extension period after the end of a trial, when all participants are receiving the new drug, also those who were on placebo, not only the therapeutic effect should be monitored for several years, but the side effects also. This is also important for the approval of the drug. This task is not so difficult if the patient data are listed in a registry which uses an active program for long-term supervision of patient health.

Dr. Korinthenberg showed a list of treatment strategies whose possible long-term side effects should be monitored. This is especially important for the chronic neuromuscular diseases and when drugs with only moderate therapeutic effects are used. Some unexpected side effects may be problems with growth, osteoporosis, liver and renal function, and hypertension, which might appear years after the end of clinical trials.

Bruno Eymard (Institute of Myology, Paris, France) spoke about **Post-marketing studies using Pompe disease as a paradigm**. Pompe disease, a glycogen storage disease, caused by a mutation of the gene for the enzyme alpha-1,4-glucosidase on chromosome 17, exists in two forms with early onset in childhood and late onset in adults. The enzyme replacement therapy with Myozyme[®], developed and marketed by the Genzyme Corporation, is successful in children and easy to supervise. But in adults, the treatment is difficult to assess as the clinical development and the symptoms are more complex and Myozyme has only a modest effect.

Because of the high cost of Myozyme, the French Ministry of Health requested a post-marketing study that would clearly show a long-term positive effect of the drug not only in children but also for the more difficult to control disease in adult patients. For this reason, a French registry was set up in cooperation with Genzyme which now contains about 15 data sets of 75 Pompe patients. It is used in this study to evaluate the patients before, during and also after the treatment once or twice per year to determine the value of the treatment for the health of the patients and their life situation, but also to detect late manifesting side effects. The results of this post-marketing study should convince the health authorities in France to continue paying for the treatment.

This registry has promoted a very fruitful collaboration between the French teams involved in Pompe disease.

In his second presentation, **Francesco Muntoni** (University College, London, UK) talked about **Combination of old and new approaches. Forward look**, and started to ask whether one should do clinical studies with different potential drugs at the same time. How could one know which component of such a drug combination was responsible for any positive therapeutic or negative side effect?

As more and more approaches have to be tested in clinical trials, we will have to face the problems of combination therapies. Biomarkers for each single drug effect would be of great help and would have an effect on the outcome measures to be used in such a combination trial. And these outcome measures should also take care of personal consequences like the increasing burden families have to face when caring for their older sick children and the deterioration of the patients’s quality of life.

If half a billion euros, as offered by GlaxoSmithKline for the development of just four exon-skipping drugs are needed for only 35% of the Duchenne patients, then we will need many billions more for the more than 100 of these personal drugs to treat 45% more patients. But the remaining 20% will need other drugs, mostly pharmacological ones, which are not mutation-specific. We need to help these patients, too. And although Duchenne dystrophy is the most frequent of the neuromuscular diseases, it is not the only one. For the others, effective treatments have to be found, too.

But we do not need only immense amounts of money,

we need many more researchers in more countries than now who will help us to develop new drugs and more effective methods. And for those of our patients, who have to wait many years more, we need better and standardized medical management procedures not only in our developed countries but in the rest of the world also. And all patients, their families and their doctors, wherever they live, need access to up-to-date information so that none of them is left behind when the first and then many more effective drugs will be there in a few years.

Discussion:

Robert Griggs: If complete restoration of dystrophin in all muscles could be obtained in all muscles, this might be sufficient for approval without placebo control. *Francesco Muntoni:* But 30% of normal dystrophin level might be enough for good muscle function. I am confident that we will be able to demonstrate this correlation between dystrophin level and muscle function also in Duchenne boys as it exists in dystrophic mice and dogs.

Petra Kaufmann: The key for our success is the partnership and commitment of researchers, clinicians, industry, and patient organizations, our stakeholders. Continuous data collection in registries is crucial to find out what happens with and without treatments. This is important for approval and funding by NIH. *Bruno Eymard:* Patients should not be lost when they move or change their doctors. Good communication between clinical centers is essential.

Victor Dubowitz: There is no substitute for common sense! One may also think to start a steroid therapy with daily application for three months, then switch over to intermittent application, the side effects will be lower, and if one continuous for a long time, one could perhaps reduce the dose slowly until the treatment could even be stopped completely. We should look at the experience with other diseases like rheumatism where steroids are also used and

side effects have been studied for a long time already. *Robert Griggs:* Our study is long-term and we will monitor the side effects for a long time too. When we find the therapy with the least side effects, we will try to get a consent agreement and then recommend this optimal treatment.

Kate Bushby: Every patient should have the opportunity to take part in a trial that is appropriate for him or her. And all patients should understand that they might get a placebo, but they should also know that all will get the standard of care including steroids and long-term follow up.

Elizabeth Vroom: We get requests from patients in countries without trial centers who even would like to emigrate and move to us! And there are patients whose exon skipping drug might not be developed for a very long time or even never. They should have access to pharmacological trials.

Francesco Muntoni and others: The results of about 30% of all trials are never published, sometimes because companies do not wish to give away their data. But all trials really should be published even those which were badly designed and therefore had negative results. We could learn from those mistakes.

A representative of Genzyme Corporation: Our registry for Pompe patients is now 20 years old, to maintain and to work with it costs us millions of dollars per year. Long-term follow-up of patients with the help of registries is important for the approval process and also for the decision of who will later pay for the treatment. The 6-minute-walk test with Pompe patients proved the clinical benefit of Myozyme. The Genzyme drugs for Fabry disease took 15 years and for Gaucher's disease 8 years until an optimal treatment was found after the clinical trials were done, and the regulators accepted the follow-up data as a proof of benefit. You cannot obtain such results from clinical trials alone.

Breaking down the barriers between stakeholders.

Closing keynote lecture by *Volker Straub*, TREAT-NMD Coordinator, Newcastle University, UK.

Dr. Straub started his closing lecture by asking, who are "our" stakeholders, the different groups of people with, in our case, one common interest, namely to find ways to effective therapies for neuromuscular diseases: the patient organizations, the scientists, the clinicians, the large and small pharmaceutical companies, the regulators, and the funding agencies. Although they all wish to understand each other and to work together towards the common goal, there are barriers between them, because they have their individual interests which caused them to develop their own knowledge based on their professional training and qualification, their own specialized language with difficult words, and their own ways of thinking about what is important and what is not. These barriers must be taken seriously, they can slow down progress, and that is the reason why TREAT-NMD, our European Network of Excellence, sees it as its main task to bring our stakeholders together and "herd" them into the one direction that will bring us therapies for Duchenne muscular dystrophy, spinal muscular atrophy, and for many other of our still untreatable diseases.

We have to bring these barriers down. We must improve the way we talk to each other: the scientists must learn to explain their research to the patients and their families in a language they can understand, which is not easy, as not everybody has learnt how science works. The language barrier is closely related to cultural differences between stakeholders. The concept of disease and treatment is often fundamentally different between different cultures and on different continents.

False expectations can also be a barrier. Ten years to develop a new drug might seem to be normal for a scientist, while a family with a Duchenne boy needs it "tomorrow" before it is too late. Expectations can be influenced by press releases, by publications, by general hype and excitement about new technologies and discoveries. Therapeutic misconception or hope that there will be improved treatment strategies in the near future is something that doesn't just affect patients and families, but also clinicians, scientists, industry and funding bodies.

Dr. Straub pointed out that people are exchanging knowledge at an ever-increasing pace, which is having a

fundamental impact on our working lives and lifestyles. The German philosopher *Hermann Lübbe* called that “Gegenwartsschrumpfung”, the shrinking of the present. The future for everything arrives faster than ever, and we hope effective therapies will come to us faster, too.

To bring down the barriers, communication between the stakeholders must be intensified and there is a willingness to do this. It is for us together to decide in which direction we want to move. The modern communication methods, like e-mails, allow worldwide correspondence at

Some concluding words

by *Kate Bushby*, TREAT-NMD Coordinator, Newcastle University, UK

“We know that the first clinical trials with a new drug or procedure must provide a *proof of concept*, a very cautious experiment to find out whether a new idea really works. This meeting was in a way also a *proof-of-concept* trial of our new idea: that collaboration is key to the success of translational research. Like all scientific meetings, ours had presentations and posters on its program. But we also were keen to explore a new way of working for such a large international conference: with open discussions between specialists on panels and the many specialists in the audience.

We believe that this concept did make the meeting successful so that in reality many *barriers* between the laboratory and the clinic would be *brought down*, so that we would find new ways to bring therapies for neuromuscular diseases rapidly to the patients.

I would say that this *proof-of-concept experiment* gave very positive results: our 350 participants from 26 countries actively worked together. Many of them had submitted their questions and ideas in writing before the conference which were then mostly answered by the panel members of the different sessions. New questions and ideas appeared and were discussed and some important themes emerged.

Patients wherever they live should have the opportunity

the speed of light, and they make now even virtual meetings possible in which one can actively participate from a computer at home.

Dr. Straub ended his lecture by saying that this meeting with its unique mixture of presentations and panel discussions has shown how important it is to meet personally and talk face-to-face, and that there is no better way to bring down the barriers and to come to a consent on the many ways of how to proceed into the right direction towards a therapy for a long life of our patients.

to take part in trials and have hope that a drug or other therapy would help them immediately or later. Patients and families, wherever they live, should get access to the most up-to-date information about research on their disease, on the best care to make their life meaningful, and to the drugs for their disease when they are there even if they cost a lot of money.

Registries need to be maintained for the future to learn everything about the diseases, and about the long-term side effects of new therapies so that it becomes known what may happen in the future. Then next-generation drugs can be developed to optimize treatments more and more. And the health authorities in all countries should accept their responsibility to take care of all their people with rare diseases.

All of these important points can only be driven home if the collaborative spirit that was embodied in this meeting is still applied between all the stakeholders in the years to come.

So this meeting will be known as a milestone that we need to follow up. TREAT-NMD will do everything that we can to see that this will be possible. And I thank you all, who made this meeting so successful, for your continued efforts to work together *without barriers* to effective therapies for all our patients.”

Thank you.

You can see this report on the internet at www.duchenne-information.eu as well as my earlier reports on research for Duchenne muscular dystrophy and some interviews. If you wish to receive all my future reports in English, Spanish or German as soon as they are ready, please send me your e-mail address for inclusion in my mailing lists which already contain more than one thousand addresses.

I am thanking TREAT-NMD and the American Parent Project Muscular Dystrophy, PPMD, for financial support. Here is the address of TREAT-NMD, our neuromuscular network: Institute of Human Genetics, University of Newcastle upon Tyne, NE1 3BZ, UK, Tel.: *44-191-241-8605, internet: www.treat-nmd.eu.

I sent most of the speakers at the Brussels conference the first version of my summaries about their work and asked them to suggest changes and additions where it was necessary. Almost all of them did so, and this means that there

should not be any or very few mistakes left. I would not have been able to write this report without this help. All my thanks go to them.

I am sending all of you my best regards from my winter working place in the Black Forest of Germany, about 30 kms east of Freiburg.



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