CDKL5 FORUM

LONDON | 5-6 OCT 2015

Wellcome Trust Conference Centre

London, 5th-6th October 2015

MEETING REPORT

Hosted and organised by

loulou FOUNDATION

In collaboration with

International Foundation For CDKL5 RESEARCH
HOPE • LOVE • CURE

Research and Support
CDKL5 UK
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INTRODUCTION

On October 5th and 6th 2015, the LouLou Foundation hosted the inaugural CDKL5 Forum meeting at the Wellcome Trust Conference Centre, London, in partnership with CDKL5UK and the International Foundation for CDKL5 Research (IFCR). A multi-disciplined gathering of over 90 leading scientists, clinicians, CDKL5 patient advisory groups and biopharma executives attended the meeting which was convened to explore ways to advance the study of CDKL5 disorder at the genetic and molecular level and to discuss the development of possible therapeutic avenues.

The CDKL5 Forum brought together committed experts and interested parties to share current research in CDKL5 and stimulate peer group discussion in an environment of openness, knowledge sharing, collaboration and creativity. On the first morning the vital patient and parent perspective was shared and the current research and understanding of CDKL5 was reviewed as well as some new techniques in genetic research. This was followed in the afternoon by four multidisciplinary workshop groups, each addressing the same questions under the theme of 'Challenging Convention to find a Cure for CDKL5'. On the morning of the second day the workshop moderator from each group presented their group's findings and recommendations for future success in understanding and tackling CDKL5 deficiency, which were discussed with the audience. A short list of priorities for the way forward was drawn up with recommendations for basic science, therapeutic approaches, new treatments and clinical translation.

CDKL5 Disorder is a rare monogenic condition currently estimated to affect some 1,200+ children worldwide, but with the estimate of prevalence growing because of increased genetic testing. CDKL5 is a serine-threonine kinase whose deficiency causes a neurologic disorder first described clinically in 2004 as an early-onset variant of Rett Syndrome. Symptoms include severe learning difficulties, seizures, sleep disturbance, and decreased visual acuity.

The Loulou Foundation is a non-profit UK foundation established in 2015 and committed to further the development of research to better understand the condition and to actively pursue multiple approaches to achieving rapid treatments and cures for this devastating neurogenetic disorder.

CDKL5 FORUM FACULTY

Forum Chairman

Sir Colin Blakemore	Professor of Neuroscience & Philosophy, Oxford University, Director, Centre for the Study of the Senses, UK; Trustee, Loulou Foundation

Speakers

Prof Simon Fisher	Director, Max Planck Institute for Psycholinguistics, Netherlands
Dr David Frame	Member of IFCR Scientific Advisory Board, Clinical Pharmacist & Assistant Clinical Professor, University of Michigan, USA
Dr Omar Khwaja	Head of Rare Diseases, Roche Pharma Research and Early Development, Switzerland
Dr Charlotte Kilstrup-Nielsen	Associate Professor, Laboratory of Genetic and Epigenetic Control of Gene Expression, University of Insubria, Italy
Dr Nicoletta Landsberger	Associate Professor, Dept. of Technical and Applied Science, University of Milan, Italy
Mr Martyn Newey	Consultant Orthopaedic Surgeon, Trustee of CDKL5UK, Leicester General Hospital, UK
Prof Jan Nolta	Director of the Stem Cell Program, Institute for Regenerative Cures, University of California, USA

Workshop Moderators

Prof Dario Alessi	Professor of Signal Transduction, Director of MRC Protein Phosphorylation & Ubiquitylation Unit, University of Dundee, UK
Prof John Christodoulou	Professor of Genetic Medicine, University of Sydney, Australia
Prof Peter Kind	Director, Patrick Wild Centre, University of Edinburgh, UK
Prof James Wilson	Professor of Medicine, Director, Gene Therapy Programme & Orphan Disease Centre, University of Pennsylvania, USA

Organiser

Dr Fran Sivers	Berrymede Associates; Consultant to Loulou Foundation

Collaborators

Carol-Anne Partridge	Founder & CEO, CDKL5 UK, UK
Karen Utley	Treasurer & Co-Founder, International Foundation for CDKL5 Research (IFCR), USA

DELEGATES

Dr Kate Adcock	Head, Neuroscience & Mental Health, Medical Research Council, UK
Dr Hayley Archer	Consultant, Institute of Genetics, University Hospital of Wales, UK
Dr Mark Bailey	Senior Lecturer (Human & Molecular Genetics), University of Glasgow, UK
Dr John Baird	Associate Director, Project Management, PTC Therapeutics Inc. USA
Dr Lucas Baltussen	Research Associate, Crick Institute, UK
Dr James Baumgartner	Surgical Director, Comprehensive Paediatric Epilepsy Centre, Florida Hospital for Children, USA
Dr Tim Benke	Associate Professor Paediatrics, Neurology & Pharmacology University of Colorado, USA
Prof Rose-Mary Boustany	Head, Division of Paediatric Neurology, American University of Beirut Medical Centre (AUBMC), Lebanon
Dr LaTese Briggs	Director, Centre for Strategic Philanthropy, Washington, USA
Dr Enrique Carrazana	Chief Medical Officer, Acorda Therapeutics, USA
Dr David Cavalla	Chief Scientific Officer, Healx, UK
Wayne Channon	Chairman, Cells4Life, UK
Dr Richard Chin	Consultant Paediatric Neurologist & Director, Muir Maxwell Epilepsy Centre, University of Edinburgh, UK
Dr Elisabetta Ciani	Associate Professor of Physiology, Department of Biomedical & NeuroMotor Science, University of Bologna, Italy
Prof Angus Clarke	Clinical Professor Institute of Cancer & Genetics, University of Cardiff, UK
Dr Stuart Cobb	Reader, Institute of Neuroscience & Psychology, University of Glasgow, UK
Simon Collins	Regional Vice President, Amicus Therapeutics Inc. UK
Prof Michael Cousin	Chair of Neuronal Cell Biology, Head of Pre-Clinical Research Muir Maxwell Epilepsy Centre, University of Edinburgh, UK
Dr Jeff Drew	Chief Scientific Officer, Stabilitech, UK
Dr James Eubanks	Associate Professor, Division of Neurosurgery, University of Toronto, Canada
Dr Michela Fagiolini	Assistant Professor of Neurology, Boston Children's Hospital, USA
Dr Christos Fokoloros	Researcher, Faculty of Medicine, University of Crete, Greece
Dr Claudia Fuchs	Researcher in Neuroscience, Department of Biomedical and NeuroMotor Science, University of Bologna, Italy
Dr Maurizio Giustetto	Researcher in Neuroscience, University of Turin, Italy
Dr Steven Gray	Assistant Professor, Department of Ophthalmology University of North Carolina, USA
Dr Tim Guilliams	Chief Executive Officer, Healx, UK
Dr Eveline Hagebeuk	Paediatric Neurologist, SEIN, Netherlands
Dr Nico Hansen	Partner & CIO, Apax Partners, UK

Prof Giles Hardingham	Professor of Molecular Neurobiology, Centre for Integrative Physiology, University of Edinburgh, UK
Dr Ralph Hector	Research Associate, Centre for Neuroscience, University of Glasgow, UK
Prof Takao Hensch	Professor of Molecular & Cellular Biology, Harvard University, USA
Dr John Isaac	Head of Neuroscience & Mental Health, Wellcome Trust, UK
Michael Jasulavic	Healthcare Analyst, HBM Partners, USA
Dr Michael Johnson	Deputy Head, Centre for Clinical Translation, Imperial College, UK
Dr Vera Kalscheuer	Head of Chromosome Rearrangements & Disease Group Max Planck Institute for Molecular Genetics, Germany
Dr Walter Kaufmann	Member IFCR Scientific Advisory Board, Director, Rett Syndrome Program, Boston Children's Hospital & Harvard Medical School, USA
Dr Farid Khan	Director, Lumophore, UK
Dr Maria Kinali	Consultant Paediatric Neurologist, Imperial College, UK
Dr Robin Kleiman	Director of Preclinical Research, Boston Children's Hospital, USA
Dr Nicholas Larkins	Director, AKL Research & Development, UK
Dr Jocelyn Le Blanc	Research Associate, Boston Children's Hospital, USA
Dr Helen Leonard	Associate Professor, Telethon Kids Institute, University of Western Australia
Dr Rhiannon Macrae	Scientific Adviser, Broad Institute of MIT & Harvard, USA
Dr Laura Mamounas	Program Director, Neurogenetics, National Institute of Neurological Disorders & Stroke, NIH, USA
Prof Oscar Marin	Director, MRC Centre for Developmental Neurobiology Kings College London, UK
Prof Nicholas Mazarakis	Lucas-Lee Chair of Molecular BioMedicine, Head of Gene Therapy, Imperial College London, UK
Dr David Millar	Lecturer & Research Fellow, Institute of Cancer & Genetics, University of Cardiff, UK
Monique Molloy	Chief of Staff, Gene Therapy Centre, Admin. Director, Orphan Disease Centre, University of Pennsylvania, USA
Dr Alysson Muotri	Associate Professor, Department of Paediatric & Cellular Molecular Medicine, University of California, USA
Dr Heather Olson	Consultant Neurologist & Epileptologist, Boston Children's Hospital, USA
Dr Sunny Philip	Consultant Paediatric Neurologist & Epileptologist, Birmingham Children's Hospital, UK
Dr Tommaso Pizzorusso	Associate Professor, Institute of Neuroscience, University of Florence, Italy
Dr Kate Roche	Researcher, Harvard Medical School, USA
Prof John Rouse	Professor of Chromosome Biology, University of Dundee, UK
Dr John Sinden	CSO, ReNeuron, UK
Dr Sally Till	Research Associate, Patrick Wild Centre, University of Edinburgh, UK
Prof Nicholas Tonks	Deputy Director, NCI-Cancer Centre, Cold Spring Harbour Laboratory USA

Dr Stefania Trazzi	Research Associate, Department of Biomedical Neuromotor Sciences, University of Bologna, Italy
Dr Chris Trotta	VP of Biology, PTC Therapeutics Inc. USA
Dr David Tukey	Research Analyst, Everpoint Asset Management, New York, USA
Dr Sila Ultanir	Group Leader, The Crick Institute, UK
Dr Clare Wilson	Consultant Opthalmologist & Founder, RetVas, UK
Prof David Wyllie	Professor of Ion Channel Physiology & Pharmacology, Director Centre for Integrative Physiology, University of Edinburgh, UK
Dr Zhaolan (Joe) Zhou	Associate Professor, Department of Genetics, University of Pennsylvania, USA

CDKL5 FORUM PROGRAMME

Monday 5th October – Wellcome Trust Conference Centre

08.30	*Forum registration and refreshments*	
09.00	Welcome and Introduction	Chairman Sir Colin Blakemore
09.15	CDKL5 - The Patients' Perspective	Mr Martyn Newey Trustee, CDKL5UK
09.45	Molecular and Genetic Aspects of CDKL5	Prof Nicoletta Landsberger University of Milan & Prof Charlotte Kilstrup-Nielsen University of Insubria
10.15	Updates in CDKL5 Research and Clinical Progress	Dr David Frame, IFCR Scientific Advisory Board
11.00	*Coffee Break*	
11.30	Genetically Engineered Stem Cells as Proposed Therapeutics for Diseases of the CNS	Professor Jan Nolta, University of California
12.00	Development of Treatments for Children with Rare Disease: A View from Industry	Dr Omar Khwaja Roche
12.30	Chairman's summary	Sir Colin Blakemore
13.00	*Lunch*	
14.00	Innovation Workshops: 4 Multidisciplinary Groups 'Challenging Convention to find a Cure for CDKL5'	*Moderators* Prof Dario Alessi Prof John Christodoulou Prof Peter Kind Prof James Wilson
17.30	*First day close*	
19.30	*Reception/Dinner at the Royal College of Physicians*	
	Guest Speaker: Prof Simon Fisher, Director Max Planck Institute for Psycholinguistics 'Molecular Windows into Speech and Language Disorders'	
22.00	*Close*	

Tuesday 6th October – Wellcome Trust Conference Centre

09.00	Meeting re-commences Introduction to Workshops Feedback	Chairman: Sir Colin Blakemore
09.15	Workshops Feedback	Prof Dario Alessi Prof John Christodoulou Prof Peter Kind Prof James Wilson

11.15 *Coffee Break*

11.30 General Discussion

12.40 Summarising Meeting Outputs and Planning for the Future

12.55 Final Remarks and Conclusions

13.00 *Meeting close*

Dr. Zhaolan Zhou

MEETING PROCEEDINGS

Welcome and Introduction

Professor Sir Colin Blakemore, Forum Chair

Sir Colin Blakemore

Sir Colin Blakemore welcomed everyone to the meeting and set out the aims and objectives of the CDKL5 Forum: To foster a community of openness, knowledge exchange, collaboration and creativity, in order to share the results of current research and stimulate peer group discussion and to identify the way forward for CDKL5 research from basic science through to developing treatments and clinical trials.

He expressed his delight at the impressive list of attendees, which included 92 participants from 13 countries, 25 universities, 9 academic institutions, more than 10 biopharmaceutical companies and 10 parents of children with CDKL5 disorder from the leadership of the patient advocacy groups.

Sir Colin said "it is a priority to understand the disease and alleviate suffering… for this we need to think outside the box and be creative". As a neuroscientist Sir Colin remarked, "I work on brain development, particularly visual development, and it is clear that the mutations of CDKL5 disturb neural development and processes in the brain… we should remember that for other conditions the continuing plasticity of the nervous system offers the possibility of new approaches to remediation and reversal, even in older children… let us maintain hope and an open mind".

CDKL5 - The Patients' Perspective:

Mr Martyn Newey, Trustee, CDKL5 UK

Mr Martyn Newey

Martyn Newey's youngest daughter Ellie was diagnosed with CDKL5 in 2010 when she was 13 years old. He defined CDKL5 as an X-linked neurodevelopmental disorder, and went on to summarise the disorder's multiple symptoms and explain the patient and parent perspective. "Parents want CDKL5 to be recognised as a distinct condition and not as a variant of Rett syndrome. We would like publications of CDKL5 research to reflect this."

He told the Forum, "CDKL5 affects the whole family." The sort of questions CDKL5 parents ask don't have easy answers, and it may occasionally be the case that a clinician doesn't know the answer to a parent's question. However, while it's actually OK for the clinician to say "I don't know", what's important is what the clinician does then.

Parents will still have expectations and it is essential that the clinician understands and meets these. "I don't know" followed by "don't worry about it" is never good enough.

Typical Questions asked by CDKL5 Parents

- Why is my child having seizures and why won't they stop?
- Are these funny turns epilepsy?
- Why is my child awake all night and what can I do about it?
- What can we do to help with feeding and bowel function?
- Why is my child crying and distressed and what can I do?
- What is the best therapy to help them try and walk?
- What is going to happen in the future?

"Parents have a lot of support and information gained through the fantastic networks such as the Facebook 'CDKL5 Parents Support Group'. But clinicians need to engage with and talk more to the parents, not just look at their child… parents need support and knowledge to provide the right care".

Newey described what families live with to put a real face to the condition. He explained that CDKL5 is incredibly varied in the type and severity of symptoms and this produces questions that are not always easy to answer.

- Some patients can have 3-5 seizures per day – we need to understand more about CDKL5 related seizures and how to treat them.

"You learn just how long 30 seconds is when you watch someone you love having a seizure"

- Many patients have recurrent episodes of sleep disturbance. This can be a full 72 hour long 'all night party' with no respite and parents are constantly worried and sleepless themselves.
- CDKL5 children can have a variety of gastrointestinal symptoms that cause their own range of problems, including gastroesophageal reflux, feeding difficulties, and constipation.

"This places enormous pressure on family life and parents need support through better integrated care."

As a parent of a child with CDKL5, Newey set out his wish list:

- For Professionals to be better informed about CDKL5
- For Clinicians to meet expectations
- Keep an "open mind" – there is still much to understand
- Most urgently, we need a holistic approach to care

Martyn told the Forum, "We want and need you to succeed. However, we as parents also want to see better collaboration between researchers. We would like to see more "Centres of Excellence" established, particularly in the UK, to provide the necessary coordination and integration of care that our children need and deserve."

Commenting later on this presentation, one of the attendees said in their feedback:

"I must emphasize that I found Martyn Newey's presentation to be highly effective and extremely moving – it is something I will never forget. Often we see scientific problems in the abstract, but this really gave the research we are trying to do a human face. It was highly motivating".

Molecular and Genetic Aspects of CDKL5:

Dr Nicoletta Landsberger, Milan University &
Dr Charlotte Kilstrup-Nielsen, Insubria University

Dr Nicoletta Landsberger

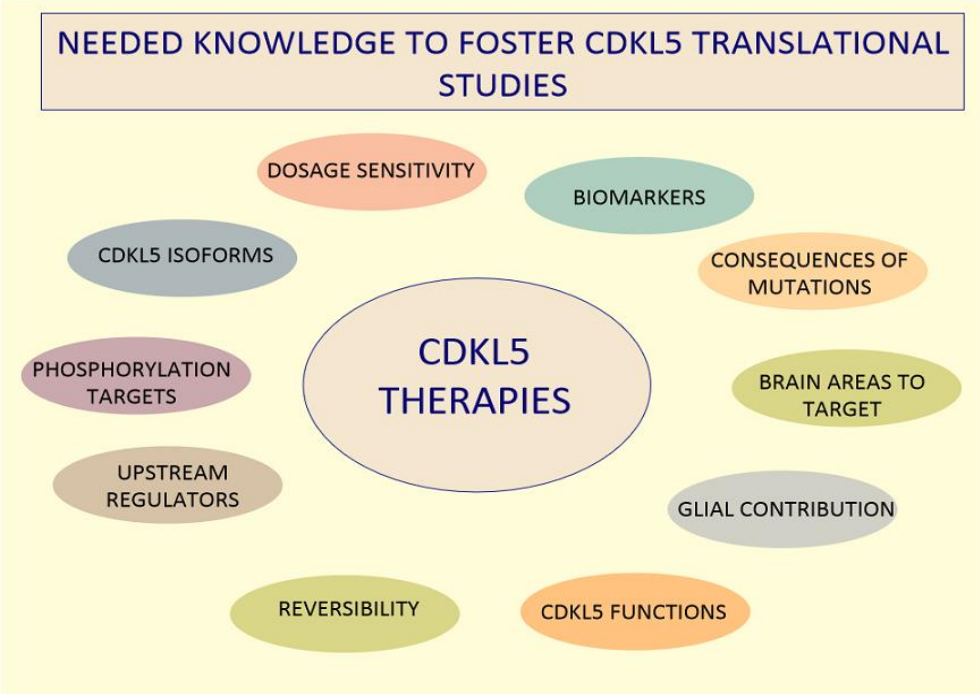
Drs Landsberger and Kilstrup-Nielsen have contributed greatly to the wider knowledge of CDKL5 and related neurodevelopmental disorders by identifying the predominantly expressed set of splice isoforms in rodent and human brains, and by mapping the gene expression profile during neurodevelopment, as well as across neuronal compartments. Their work has shown that CDKL5 is an activity dependent neuronal kinase whose subcellular distribution and levels are very closely related to neuronal activation. The kinase also appears to have a role in neuronal maturation, dendritic arborization, synaptic plasticity and learning and memory.

Dr Charlotte Kilstrup-Nielsen

The presentation provided a critical review of the current CDKL5 literature to identify gaps in knowledge that need to be filled. Landsberger noted that the level of CDKL5 changes quite a lot depending on tissue but questioned "is it upregulated in all parts of the brain?" She asked whether future research should focus on the CNS (especially the forebrain), and also wondered about the expression of CDKL5 in glia and how the role of CDKL5 in glia vs. neurons should be clarified. She explained that to accelerate successful treatments for CDKL5 deficiency a number of key-points could be addressed:

- A reliable biomarker for CDKL5 needs to be found so that treatment can be monitored. Gene expression profile studies might help
- Is the disease reversible and what is the time window for treatment?
- CDKL5 over-expression causes some changes in neuronal morphology: for protein or gene therapy to be successful we need to know the target dose of CDKL5 required.
- We need to know more about the diversity of patient phenotype and relate this to the pathogenesis of the disease.
- It would be useful to understand the functional consequences of the mutations in CDKL5.
- One of the big questions is where and when does CDKL5 exert its kinase activity? Is it in the cytoplasm or nucleus?
- It is highly important to search for the phosphorylation targets and any modulators of the CDKL5 kinase
- The current mouse models showed a clear phenotype but do not have seizures, so additional animal models could be useful as well.
- We need to know what CDKL5 does in dendritic spines and to assess whether the role of CDKL5 in spines might be relevant for gene and protein therapy approaches.
- It would be good to have a better understanding of the role of CDKL5 in all compartments of the cells.

Landsberger and Kilstrup-Nielsen summarised the knowledge sought to foster CDKL5 translational studies:



They finished the presentation by highlighting the importance of understanding the transcriptional consequences of the loss of CDKL5 and by suggesting a new exon numbering scheme for the CDKL5 gene.

Updates in CDKL5 Research and Clinical Progress:

Dr David Frame, IFCR Scientific Advisory Board

Dr David Frame

Dr Frame thanked the Loulou Foundation for its efforts on behalf of the CDKL5 community. He is the father of a 9-year old girl Kiera, with CDKL5. He explained how he helped to start the research programme for the IFCR which includes new and available mouse models, a programme at the Muotri Laboratory in USCD to culture iPSC (induced pluripotent stem cell-derived) neurons to transplant into patients, research into gene expression impairment in human CDKL5 neurons and correlating EEG recording with seizure activity measured with a multi-electrode array on iPSC-derived neural cells. Dr Frame explained how iPSC models will tie in with testing several therapeutic approaches currently under consideration. "These include a range of therapies that are being currently being researched" including:

- Gene Therapy Vectors
- Protein Therapy
- PTEN (Phosphatase and Tensin homolog) Inhibitors
- ADHD (attention deficit hyperactivity disorder) medications/Dopamine antagonists.

Frame explained that Glycogen Synthase Kinase inhibitors (GSK-3β) have recently proved to be a promising area. GSK-3β is an inhibitory regulator of neurite outgrowth, synapse formation, neurogenesis and survival of newly-generated neurons and, according to two recent papers by Fuchs in Neurobiology of Disease "..could be a significant breakthrough in treatment of CDKL5". However there is a problem with toxicity with some current GSK-3β inhibitors. We may need to also develop new ones with better specificity.

In addition one of the IFCRs current funded projects is to examine CDKL5 phosphorylation targets such as amphiphysin 1, MeCP2 , DNA Methyltransferase 1 and NGL-1.

The IFCR is also doing a lot of work in developing the clinical aspects of CDKL5. Priorities here include:

- The CDKL5 Centres of Excellence, to help develop physician expertise and establish guidelines and standards of care.
- Establish an International Database Registry for CDKL5 (working with Helen Leonard of the Telethon Kids Institute in Australia)
- Involvement in the NIH natural history project for studying Rett syndrome, MECP2 Duplication disorder, and Rett-related disorders.
- Participation of CDKL5 patients and parents in the Rare Epilepsy Network to examine CDKL5 epilepsy and seizures in depth and establish clinical and treatment guidelines.

Frame completed his talk by referring to future clinical areas of interest associated with CDKL5, listed in the table below, which will contribute to the breadth of knowledge about the disorder.

Future Clinical Areas of Interest:

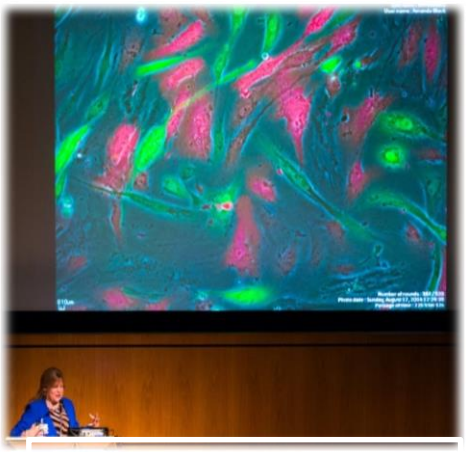
- GI system
- Immune system
- Visual aberrations/Cortical Visual Impairment (CVI)
- Movement abnormalities
- ADD/ADHD features
- Autonomic Dysfunction
- Augmentative communication
- Cardiac system
- Metabolomics

Professor Jan Nolta

Genetically Engineered Stem Cells as Proposed Therapeutics for Diseases of the CNS:

Professor Jan Nolta, University of California

Professor Nolta provided an excellent overview of the impressive work being carried out at UC Davis Institute for Regenerative Cures. Using examples from some of the 18 therapies that are currently in their pipeline she explained how stem cell and gene therapies could be used to treat CNS and immune system conditions such as bubble baby disease and San Filippo Disease (mucopolysaccharidosis IIIA or MPS-IIIA). Nolta went into detail about using Mesenchymal Stem Cells (MSCs) engineered to produce Brain-derived Neurotrophic Factor (BDNF) as a potential treatment for Huntington's Disease (HD) and how this approach could be applied to other diseases. Professor Nolta told the forum, "Survival and function of striatal neurons is dependent on BDNF… mutant Huntington protein blocks production of BDNF at the RNA level… Levels of this trophic factor are significantly reduced in the brains of HD patients… Dey et al, in Gary Dunbar's lab, showed that murine MSCs engineered to over-express BDNF slowed the progression of HD in a transgenic mouse model." Nolta explained that the BDNF therapy is now heading toward clinical trials and eventually a viable treatment for Huntington's disease.



Slide Showing Stem Cell Activity

She also explained how new strategies involving artificial transcription factors (essentially DNA binding proteins) could change the way a part of the genome is expressed and prevent some diseases by gene silencing.

She suggested the following therapeutic stem cell and gene therapy options for treating CDKL5:

- Gene Therapy: Adding a normal gene copy to restore function
- Neuroprotection: Promoting endogenous neurogenesis
- Gene Modification/Correction: Silencing a dominant mutant allele
- Cell Replacement Strategies
- Transplantation of healthy progenitors capable of neuronal differentiation
- Transplantation of gene-modified patient iPSC-derived neurons
  (Muotri lab- CDKL5, Thompson lab- HD; progress made weekly)

Developments of Treatments for Children with Rare Disease: A View from Industry:

Dr Omar Khwaja, Roche

> "I used to be a paediatric neurologist, ran a Rett program in Boston, and I can tell you CNS neurological disorders in children are some of the most under-served… The more time I spend, the more I see drug development in children is like this Rubik's Cube… a time contextual metaphor and very complex… how can we get therapies into the brain in a way that works for children?"
>
> Dr Omar Khwaja

Dr Khwaja provided an industry view on rare diseases and gave his thoughts on how to approach drug development and delivery in this important area. He asked "Why is a large biopharmaceutical company like Roche taking an interest in rare diseases?" "I want to try and answer that question and maybe introduce some ideas to frame the workshops…

Dr Khwaja explained that industry sees the rare disease market in a different way to that of a blockbuster drug market. "The company perception of risk is different for rare diseases", he said. "Scientific and genetic diagnostic advances show more than 250 new rare diseases discovered every year (there are currently over 7,000 rare diseases identified) and this translates to:

- An unmet medical need of some 300 million people worldwide with a rare disease
- 95% of rare diseases with not a single FDA approved drug treatment and
- More than 50% of rare diseases affect children with a high fatality rate of around 30%."

He explained the drive behind biopharmaceutical companies. Pharmaceutical companies want to ensure high confidence early on and as much de-risking as possible; genetic diseases offer this because of target validation and good foundation work. If we then couple this with next generation sequencing solutions, diagnosis is made easier and more robust and overall this provides a good basis for commercial confidence.

Khwaja said “I hate to talk about the commercial aspects but it is important as it gives confidence. Rare disease is one of the few growth areas in pharma… the rare disease market is well reimbursed, has funding, good recruitment for clinical trials, samples and also financial incentives… overall regulatory success for rare disease therapy is 93% compared to 89% for non-rare diseases.”

Dr Khwaja explained what is required for success including early engagement with payers, regulators, HTA and good investigators. Having clear endpoints, outcomes and biomarkers is another good idea along with development of clinical and patient networks. It seems that building belief and good science around a disease such as CDKL5 might attract the right response.

Actions needed for success?

- Early patients and foundation engagement
- Build or leverage existing clinical and patient networks
- Access and build registries
- Promote natural history studies
- Front load juvenile toxicology studies
- Invest in Phase1b/2a studies
- Aim for “quick-kill” designs with clear go/no-go criteria
- Constantly maintain and develop patient and clinician relationships
- Focus on high quality sites and investigators
- Reframe CMC hurdles
- Engage early with regulators, payers and HTA
- Innovate in endpoints, intermediate outcomes and biomarkers

Khwaja completed his talk by saying “rare diseases begin and end with the patient, we need to be lean and focus on the patient and innovation… Doing now what patients need next”.

Dr Omar Khwaja

Molecular Windows into Speech and Language Disorders:

Prof Simon Fisher, Director, Max Planck Institute for Psycholinguistics, Netherlands

Professor Fisher gave an interesting talk during the dinner about how some language and speech disorders have a genetic basis. He began by quoting Charles Darwin, "*Language is an art like brewing or baking".* Fisher said: "We have an instinct to acquire an art; without any formal tuition we learn thousands of words, which we can assemble into limitless meaningful combinations. We communicate these to other people by converting streams of thought into sound, and back again". He explained that some children have problems with this because they have a developmental language disorder, which tends to cluster in families and so suggests a genetic basis.

Over the last 15 years some of the genes that are important in this type of disorder have been identified. Professor Fisher cited the example of the KE family where three generations (15 members) suffer from the same speech and language disorder that has been linked to a mutation of the FOXP2 gene by genetic mapping techniques.

Professor Fisher then went on to relate his genetic research into FOXP2 and how it can have a profound effect on regulating lots of other different genes. It is evolutionarily ancient, present in similar form in diverse vertebrate species, where it helps regulate development and function of certain circuits in the brain. In studies of FOXP2 function, scientists are using an array of systems, from brain cells grown in the laboratory to animal models, in order to bridge gaps between genes, circuits, brains and behaviour. Studies of versions of FOXP2 found in animals and birds suggest that it is important for maintaining plasticity of a subset of brain circuits. Overall, this body of work may ultimately yield improved diagnosis and treatment of neurodevelopmental disorders involving disrupted speech and language skills, illustrating the value of gene-driven approaches. In effect, he drew a parallel with the issues surrounding CDKL5. His research and experience could help solve some of the mechanisms surrounding CDKL5 and help give a better understanding of how the disorder can be treated.

Sir Colin Blakemore & Professor Simon Fisher

Innovation Workshops: 'Challenging Convention to find a Cure for CDKL5'

Sir Colin Blakemore the Forum Chairman introduced the workshop sessions. He said "we have seen from the talks given by Martyn Newey and David Frame how understandably 'hungry' CDKL5 parents are for really rapid progress… I think that, although there is no treatment yet and CDKL5 is not fully understood, what is impressive is the amount of progress that has been made in the 11 years since the condition was first described. "I believe there is a very strong need for the establishment of Clinical Centres of Excellence in Europe based on the US model, to deliver care for patients and provide support for research". "We need to look for the best investment for rapid delivery".

Four multidisciplinary workshop groups, under the theme 'Challenging Convention to find a Cure for CDKL5' met on the afternoon of the first day. Each group was asked to address the same four topics: Basic Science, Research Tools, Therapeutics Strategies and Clinical Development. The groups were moderated by: Professor Dario Alessi, Professor Peter Kind, Professor John Christodoulou and Professor James Wilson. On the morning of the second day the workshop moderator from each group presented his group's report and recommendations for future success in understanding and tackling CDKL5 disorder. In order to prevent repetition, for each subject area Professor Alessi presented the outputs from his group, then any additional recommendations were added by presentations from Professors Kind, Christodoulou and Wilson respectively. The ensuing discussion with the audience provided a series of key messages and meeting outputs. A summary of priorities for the way forward was then drawn up with recommendations for basic science, therapeutic approaches, new treatments and clinical trials:

Basic Research

Basic science overall was thought to be a priority area, given the paucity of information on CDKL5 biology. All groups agreed that basic research could lead to strategies, such as gene editing and gene therapy as well as drug repurposing and development, which could eventually lead to effective therapies. There was agreement that basic research would need to focus on several areas:

Kinase Research

Basic kinase research on CDKL5 is required to find out how CDKL5 is regulated in the cell, how it functions (downstream targets) and what it phosphorylates (the interactome). It is essential to determine the downstream targets and the upstream modulators and to know how CDKL5 affects different tissues. A kinase inhibitor for CDKL5 would be a very valuable research tool.

The Role of CDKL5

There is a need to determine the role of CDKL5 in DNA, nucleus, spine and synapse, and the molecular basis of the disorder. Gene expression profiling is key to elucidating CDKL5 function. Key downstream pathways include AMPA up-regulation, dendritic spines (proteomics / phosphoproteomics/ transcriptomics), and developmental and geographic expression profiles.

CDKL5 may have different functions in the synapses and in the nucleus. CDKL5 appears to extend its influence to the gastrointestinal and immune systems but it was felt that researchers should prioritise study of its neurological effects, as cognitive impairment and persistent seizures are the most prevalent and distressing aspects of the disease.

Mutations

Finding out how mutations cause disease and how best to identify CDKL5 substrates is crucial, possibly using the Shokat Method or a Yeast Two-Hybrid System. A follow-on area is to determine how these mutations can be related to the phenotype of the patient. There is no current inventory of patient mutations and this might be the key to a better understanding of CDKL5.

Finding Subunits

Gel-filtration techniques could be used to potentially identify an essential subunit for activation. Also mass spectrometry screens could be used in cells/iPS cells/tissues from genetically-matched wild-type and knock-out/disease mutant/ knock-in mouse models to search for proteins that co-immuno-precipitate with endogenous CDKL5 from wild-type as possible substrates.

Substrates for Phosphorylation

A robust set of criteria validated by third-party laboratories should be drawn up to define what constitutes a genuine CDKL5 substrate. We need to generate phospho-specific antibodies to the phosphorylation site of substrates and demonstrate that these are indeed phosphorylated by CDKL5. Phospho-specific antibodies that can monitor the phosphorylation of known CDKL5 substrates such as amphiphysin or netrin could also provide valuable biomarkers. It was thought that T-loop phosphorylation of CDKL5 is likely to be vital and should be studied in detail to answer the question 'what are the stimuli that regulate CDKL5 phosphorylation and how can they be identified and utilised?' Phosphatase inhibitors could also be used on CDKL5 substrates to find sets of proteins that change in CDKL5 knock-out and wild-type mice.

Research Tools

Antibodies

It was agreed that CDKL5 has a range of functions, but a lack of knowledge about developmental expression patterns was preventing understanding of the emergence of end phenotypes. To help this lack of understanding good antibodies are needed, as a good antibody for CDKL5 has so far proved elusive. Professor Alessi suggested Nanotools or immunoGenes.com should be approached about

Prof. James Wilson; Prof. Dario Alessi; Prof. John Christodoulou & Prof. Peter Kind

producing an antibody, because of their specialist expertise. Other requirements were Myc, Flag and GFP tags N and C terminus, T-loop phosphorylated antibody and both the 115 and 107 kDa forms of the gene as well as untagged versions. It was felt that robust strategies were needed for making CDKL5 protein, which in turn could lead to the generation of better antibodies (possibly in sheep or larger animals). Any antibodies developed should be recorded online by the CDKL5 Forum, allowing the wider research community to access the knowledge.

Animal vs. Human Models

The two ways of modelling the disease are in human cells and tissues, on one hand, and in animal models on the other. Given the early stage of the science, researchers felt both avenues should be pursued. Alternative mouse models could be needed. The current mouse model of CDKL5 has the disadvantage of not showing a seizure phenotype, but the lack of seizures is likely to be due to the way mouse and human brains differ at a higher, organisational level, not because of underlying molecular pathways. The idea of developing new animal models was thought to have some merit because if seizures could be reproduced it would offer more scope for research. Also, it is very important for translation to show that phenotypes cross species. Mice have limitations that can be overcome in other species. However, developing a new animal model is expensive and has regulatory issues and husbandry costs attached to it. While a new rat model for CDKL5 is currently in development (by Edinburgh University), the team suggested that zebra fish and marmoset models might also prove worth investigating. (It was then pointed out that 3 zebra fish models for CDKL5 are already available in the UK from the Wellcome Trust Sanger Institute).

The priorities when studying human cells and tissues were put forward as a means to understand better the pathology of the disease at a histological level, and to profile the RNA and regulatory proteins. The drawback is that there is so little human tissue available for study, and how the tissue is preserved is crucial to obtaining reliable results.

Human Tissue Samples

The sensitive topic of obtaining post-mortem patient tissue samples (e.g., brain, GI, respiratory tract) was also discussed. Getting permission would not be easy but it could significantly advance research (Since then the first post-mortem patient tissues have been deposited with Harvard University). The Centres for Excellence might provide an opportunity for the collection of tissue and it was recommended that families should be approached early on, and with great sensitivity, with requests for tissue. Standards of practice for tissue handling must also be standardised at an early stage.

In addition, biobanks need to be established for CDKL5 research, to store induced pluripotent stem (iPS) cells, lymphoblast and fibroblast cell lines, with the requirement that any biobank developed should clearly differentiate between male and female cell lines.

To generate induced pluripotent cells (iPS) for applications such as drug screening, scientists will need many samples from different patients, along with CRISPR/Cas9-corrected clones. Some cautioned, however, that iPS cells do not shed light on functionality because they are functionally immature compared with the mature neurons in a human brain. However as no model is perfect, the strengths and limitations just need to be clearly outlined. This highlights why multiple preclinical models are needed.

Therapeutic Strategies

A range of potential therapeutics was discussed, including gene editing, X chromosome reactivation, read through DBS, protein replacement therapy, gene therapy and HDAC inhibitors. In addition there are pharmaceutical interventions, such as GSK inhibitors which need to be considered. Once therapies are available for testing, humans were thought to be the best model. A patient registry and associated deep phenotyping would be key to developing a model of CDKL5 for trial design and animal model testing, but especially useful for establishing biomarkers and outcome measures.

Measurable Outcomes

The outcome measures were identified as primarily neurological (basic sensory dysfunction, visual hypersensitivity/CVI, the cellular causes of epilepsy, reduction of seizures, and improvement in sleep dysfunction). Immune changes with CDKL5 were also considered as one of the more treatable aspects of the disease. There was much discussion of what might serve as a measurable endpoint and it was decided that it might lie in monitoring seizures — though the detail of exactly how to achieve this proved challenging. Clinicians in the group pointed out that EEGs are not regularly performed on patients, and that there is a sometimes a mismatch between what parents and clinicians perceive to be a seizure, and what is indicated by the data from an EEG.

Carol-Anne Partridge

Also, no two patients are alike – most have unique mutations — and this muddies attempts to define a common endpoint. In the end the group felt that seizure logs made before and after the treatment, combined with extended EEGs, might be the best way forward. There was also discussion of Visual Evoked Potentials (VEPs) as being a translatable endpoint that was independent of seizures and had already been successfully phentoyped for Rett Syndrome.

Existing Drugs

Regarding drug development, it was thought that one of the first actions should be systematic strategies to identify existing drugs that could be 'repurposed' using high through-put libraries involving primary, secondary and tertiary readouts (validated *in vitro* assays are needed, such as iPS cells and CRISPR/Cas9 corrected controls). Additionally, both iPS and mouse data could be used to help rule 'in' and 'out' drug candidates. Therapeutic strategies worth exploring for CDKL5 deficiency, include ketogenic diets and PUFA therapy, drugs that work for the MECP2 gene in Rett syndrome (such as Insulin-like growth factor 1 and FOXG1), repurposing the drug Tianeptin and the GSK-3β inhibitor being developed for diabetes. Tianeptin, an antidepressant first introduced in the 1980s, has properties relevant to CDKL5, including preventing dendritic atrophy and enhancing AMPA receptor

trafficking. Additional *in vitro* data, it was felt, would be required before Tianeptin could be used in clinical trials involving children.

This work will mostly need to be done in the public sector, said one contributor, because drug companies will only explore the repurposing of their own assets. This issue highlights the need for understanding CDKL5 function and the pathophysiology associated with it. This area of research will identify potential new pathways to target with repurposed drugs.

Drug Development

The research community needs to reach a consensus regarding the level of efficacy required from mouse models to move forward into drug development. In any resulting clinical trial, seizure control and reduction represents a valuable outcome measure, but it was recognized that there can be difficulties capturing unobserved seizures and differentiating between convulsive and non-convulsive status. Circuit-based brain rhythms like auditory evoked potential and VEPs may be able to serve as a surrogate for seizures. Sympathetic tone (measured by skin conductance) might offer a useful tool for measuring pain sensitivity and emotional responses. Also some observations have been made that show CDKL5 deficiency renders neurons vulnerable to oxidative stress and this might have the potential to deliver yet more new avenues for treatment.

Other Potential Therapies

Other potential therapies included gene therapy, but there was some discussion about whether this could deliver a treatment for CDKL5, because there appears to be a high threshold for achieving genetic correction. It is debatable whether it would be possible to reach enough targets in the central nervous system (no bystander effect). The issue of protein therapy raised questions such as: how much material is required for one treatment (CDKL5 half-life), how it would be administered (can it cross the blood-brain barrier), would over-expression be toxic and could it be an enduring cure? Many felt that the lack of standardisation in preclinical testing is a general hindrance to translation.

Even using slightly different experimental conditions, tissues and reagents can lead to results that are not easily compared with those of other teams. Another approach, genome editing, was thought to be at least 100-fold less efficient *in vivo* than cell or gene therapy. Turning the inactive CDKL5 gene back on was also discussed and was thought to offer strong potential along with strategies to re-express the CDKL5 gene on X-inactivated chromosome. A direct injection of CDKL5 expressing glial cells into the brain was also thought to have merit.

Collaboration

One proposal was to partner with a contract research organisation (CRO). Although they are expensive, they could provide a full-service evaluation of leads in animal models. This would include a pharmacokinetic pre-clinical database for the mouse and information on toxicity and efficacy. The Penn KO mouse has been deposited with Psychogenics for this purpose.

Physical therapy

Physical therapy was discussed with parents present at the meeting and they gave a positive response saying the earlier it was started the better and some excellent results had been obtained. Researchers thought this should be presented as a paper and that formal studies were required to obtain data.

Sleep Therapy

Sleep disturbance (especially REM disorders), the workshops felt, represents a major symptom of CDKL5 deficiency largely overlooked in drug development. Potential therapies for sleep disorders should be explored in mouse models. One suggested treatment for sleep disorders was Gabapentin, although this came with the caveat that response varies from patient to patient.

Dr Walter Kaufman & Dr Helen Leonard

Clinical Development

Clinical Trials and Proof-of-Concept

Once a potential therapy had been developed, it could enter the first trial stage — clinical proof-of-concept studies. Although early trials primarily measure safety, it is good to build in some indicators of efficacy as well to give an early sign that the therapy might work.

Coordination between research groups would be very valuable as well as sharing of data. Indeed the issue of sharing data, particularly in such a small field, recurred throughout the meeting. The familiar incentives not to share, mostly related to the need to publish and also to pressures, both from drug companies and foundations funding research, to protect intellectual property were highlighted. The launch of the CDKL5 Forum online portal (www.cdkl5Forum.org) at the meeting was hailed as an important step in this regard. Perhaps all of the workshop delegates realised that a spirit of cooperation is needed and embodied in a consortium of researchers that can cross-validate studies and release datasets, after publication, for interrogation by others. Many delegates championed involving regulators in the trial process as early as possible as much valuable advice and experience could be gained.

Practice Guidelines

Regarding clinical practice there was a recognized need to develop practice guidelines for CDKL5 treatment in Europe and Australia alongside those currently under development in the US. The Delphi method, taking a systematic approach using a panel of experts, could provide a valuable approach for defining standards of care. Within current knowledge a great deal more could be done to improve patient quality of life. Taking the example of cortical visual impairment, it was suggested that diagnosing whether children had dorsal or visual stream impairment would allow simple lifestyle adaptations to be introduced that would make a major difference to daily living.

Patient Registry

The registry was highlighted as a very important factor in the success of research as it would be able to map the phenotype against mutation and provide valuable base-line data. Strategies for improving the quality and accessibility of the data and how the wider community could help collate the data were discussed. Definite incidence figures for the disorder were also thought to be vital requiring natural history studies as well as a proper epidemiological study.

Summarising Meeting Outputs and Planning for the Future

Summary of CDKL5 Forum recommendations to facilitate better understanding of the fundamental biological functions of CDKL5 and the pathophysiology that results when it is mutated.

1. Encourage the standardisation of clinical guidelines and treatment pathways for CDKL5, potentially adopting a 'Delphi' approach for defining standards of care.
2. Promote the formation and maintenance of Centres of Excellence for patient care and experimental medicine
3. Form a single comprehensive registry for CDKL5 or link existing ones to gather both clinical and genetic data
4. Collaborate with other disease areas as there may be issues in common
5. Explore the possibility of a biobank for human CDKL5 tissue
6. Establish standards for preclinical models so that researchers can replicate, improve, analyse and discuss experimental evidence more easily
7. Consider the advantages of moving toward other animal models for CDKL5 research e.g., zebra fish and rats
8. Identify reagents such as kinase inhibitors, CDKL5 antibodies, and CDKL5 inhibitors and substrates
9. Engage with biopharma companies and academic institutions to share compound, kinase and antibody libraries and databases
10. Open up a link with regulatory agencies (FDA and EMEA) to discuss clinical trials and preparations leading to new therapies
11. Promote an open exchange of information where ethics will allow
12. Use the new CDKL5 portal 'CDKL5forum.org' to keep up-to-date with latest developments and promote networking

In Conclusion

The interesting presentations given on the previous day, together with the workshop feedback and all of the discussions had provided the attendees with a huge amount of information about CDKL5. This had the effect of stimulating conversation between researchers in similar fields and consequent exchange of ideas. The meeting was truly multidisciplinary bringing together professionals not only from across the globe but also from across industry, academia and learned research institutions to interact and exchange their points-of-view. A very important feature was the presence of CDKL5 parents from the patient advocacy groups who shared their personal experiences and emphasised the urgency provoked by this devastating condition. Delegates were urged to remain in contact via the 'CDKL5Forum.org' portal and to treat it as a continuance of the physical meeting for discussion and exchange of ideas.

Sir Colin as Chairman again praised the openness and co-operation across the Forum in sharing ideas and research goals for CDKL5. He emphasised that unlocking the secrets of CDKL5 and finding a cure for the disorder were priorities and that researchers, industry, regulatory agencies and parents all have a role to play and should work together. He urged everyone to attend future meetings and present any new findings.

In conclusion Sir Colin said "This meeting has provided a boost to the world of CDKL5 research and given a lot of people hope". "It's the patients that matter and that is what this is all about".